Vol. XXXVI December, 1947 No. 12

JAN 26 1948

SCIENTIFIC EDITION

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

Justin L. Powers, Editor

Committee on Publications

George D. Beal, *Chairman*
B. V. Christensen
Roy B. Cook
Robert P. Fischelis
Hugo H. Schaefer

Editorial Advisory Board

L. W. Busse
T. C. Daniels
L. W. Hazleton
E. J. Hughes
W. T. Sumerford

CONTENTS OF THIS ISSUE

Thyroid Function and the Duration of Sodium Pentobarbital Anesthesia 369
H. E. Ederstrom

The Preparation of 1-Diethylamino-2-(2,4,6-Triiodophenoxy)ethane Hydrochloride 372
Alfred Burger

The Use of Acetylation in the Quantitative Separation of Ephedrine from Procaine 373
Llewellyn H. Welsh

The Synthesis and Study of Some N-Dihydroxypropyl Derivatives 376
James W. Jones and Alfred Halpern

Chemical Determination of Synthetic Estrogens. I. Basic Data on the Nitrosophenol Reaction ... 379
Sidney Gottlieb

Comparative *In Vitro* Neutralization of Heparin 383
E. J. Mackowiak and R. D. Barnard

The Bactericidal Action of Oxygen-Liberating Substances on Oral Microorganisms. I. Studies with Fusiform Bacilli 385
Thomas C. Grubb and Marguerite A. Edwards

Chemical Investigation of *Premna Integrifolia* Linn 389
N. K. Basu and P. C. Dandiya

The Estimation of Aloin in Pharmaceuticals 391
K. G. Stone

Pharmacognostical Studies of Brahmi 393
S. Prasad

Thixotropic Mineral Gels and Their Therapeutic Possibilities 402
Herman J. Schneiderwirth and Paul W. Wilcox

Iodine Research Award Nominations Requested 406

Lubricants in Compressed Tablet Manufacture 407
J. Edward Wolff, H. George DeKay, and Glenn L. Jenkins

United States Pharmacopœial Convention Seventh Annual Financial Report 410

Notes on the Role of Hydrogen Ion Concentration and Buffer Systems in the Preparation of Ophthalmic Solutions 413
Harry W. Hind, Frank M. Goyan, and T. W. Schwarz

A Note on the Synergism of the Central Nervous System Stimulants 414
Raymond P. Ahlquist

(Continued on Inside Front Cover)

CONTENTS OF THIS ISSUE (*Cont'd.*)

A Note on the Hypnotic Principle of *Rauwolfia serpentina* 416
J. C. Gupta, Sudhamoy Ghosh, A. T. Dutta, and B. S. Kahali
Book Review 401
Index to Journal for 1947 417–423
Index to Pharmaceutical Abstracts for 1947 353–426
Advertising ..... Cover pages 2, 3, 4 and pp. I–IV

Copyright, 1948, by the American Pharmaceutical Association

BULLETIN OF THE NATIONAL FORMULARY COMMITTEE

Published by The American Pharmaceutical Association

A bimonthly information bulletin of special interest to those closely following revision trends in the official compendia. Contains preliminary drafts of new monographs and research and review articles pertaining to official drugs and drug standards. Especially valuable to teachers, control chemists, and research workers on drug standards.

Single copies, $1.00. **Annual Subscription, $5.00**

Order from

The American Pharmaceutical Association
2215 Constitution Ave., N. W. **Washington 7, D. C.**

NOTICE

PAPERS FOR SECTION MEETINGS OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

The next annual meeting of the American Pharmaceutical Association will be held in San Francisco, California, August 8–14, 1948. Members planning to present papers before any Section during this meeting are earnestly requested and strongly urged to notify the appropriate Section secretary of their intention promptly. Titles and abstracts (not to exceed 250 words) of papers should be submitted by May 1, 1948, or preferably earlier in order to insure a place on any Section program. Send titles and abstracts of papers to:

SECTION SECRETARIES

SCIENTIFIC
RAY S. KELLEY, Secretary
179 Longwood Avenue
Boston 15, Mass.

PHARMACEUTICAL ECONOMICS
E. J. IRELAND, Secretary
6363 St. Charles Avenue
New Orleans 15, La.

EDUCATION AND LEGISLATION
RALPH W. CLARK, Secretary
University of Kansas
School of Pharmacy
Lawrence, Kan.

PRACTICAL PHARMACY
ELMER M. PLEIN, Secretary
University of Washington
College of Pharmacy
Seattle 5, Wash.

HISTORICAL PHARMACY
ROLAND T. LAKEY, Secretary
Wayne University
College of Pharmacy
Detroit, Mich.

Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

JUSTIN L. POWERS, EDITOR, WASHINGTON, D. C.

VOLUME XXXVI | *DECEMBER, 1947* | NUMBER 12 | CONSECUTIVE NO. 24

Thyroid Function and the Duration of Sodium Pentobarbital Anesthesia*

By H. E. EDERSTROM

Thyroidectomy in adult female rats lengthened the duration of sodium pentobarbital anesthesia. Thyroid administration to normal rats did not change significantly the length of anesthesia. Thiouracil feeding failed to change the length of the anesthetic period. In dogs, thyroidectomy had no significant effect on the duration of pentobarbital anesthesia. Thyroid treatment of thyroidectomized dogs did not alter the length of anesthesia.

SEVERAL AGENTS have been described as antagonistic to barbiturate anesthesia, among them being drugs of the sympathetic-comimetic type. Westfall (1) reported the antagonistic effect of epinephrine on sodium pentobarbital anesthesia, and Freireich and Landsberg (2) recommended amphetamine for barbiturate poisoning. On the other hand, depressing agents, such as cold sufficient to lower body temperature, have been found to prolong the effects of barbiturate action by Raventos (3), Gaylord and Hodge (4), and Fuhrman (5).

These findings suggest that the general state of body metabolism may be a factor in determining the length of the sleep produced by this group of drugs. Rowbottom (6) implied that thyroxine could be used to counteract barbiturate toxicity, but Scarborough (7) reported that no significant change in the sleeping time of rats given pentobarbital could be produced by feeding the animals 0.03 Gm. desiccated thyroid daily. In view of these conflicting statements, it was considered that the role of thyroid in barbiturate anesthesia should be investigated further.

EXPERIMENTAL

Rats.—The methods used to alter thyroid function in rats were thyroidectomy, thyroid feeding, and thiouracil feeding. A total of 38 female and 16 male rats four months of age or older were thyroidectomized. Fourteen days or more postoperatively these were given intraperitoneal injections of 25 mg./Kg. sodium pentobarbital at intervals of one week or longer. Controls of comparable age and sex were injected with the same dose. Termination of

* Received May 8, 1947, from the Department of Physiology and Pharmacology, University of Missouri School of Medicine, Columbia, Mo.

Present address: St. Louis University School of Medicine, St. Louis, Mo.

the sleeping period was recorded when the rat voluntarily righted itself from the inverted position.

Thyroid-fed rats were given daily about 50 mg. desiccated thyroid (Parke–Davis, 50% stronger than U. S. P.) in a sugar solution administered orally. This dose of thyroid was reported by Warkentin, *et al.* (8) to increase the B. M. R. of rats 30% to 50%. After sixteen days of this regimen injections of pentobarbital were begun, and thyroid feeding continued throughout the experiment. Thiouracil-fed rats were given 0.1% of the drug in the diet for twenty-eight days prior to, and throughout the period of barbiturate injections. This concentration of thiouracil given in drinking water was found to depress the B. M. R. of rats 23.7% in fourteen days by Reineke, Mixner, and Turner (9).

Dogs.—A second series of experiments concerned the influence of thyroid function on the length of pentobarbital-induced sleep in dogs. One male and 5 female adult mongrels weighing from 5.3 to 11.7 Kg. were used. The dogs were kept indoors and fed a diet of commercial dog food throughout the course of the investigation. During the anesthetic period the animals were kept at room temperatures varying from 22° to 27°. The termination of sleep was more difficult to establish in the dog than in the rat because of the long semiconscious state persisting after the deep anesthetic effect had worn off. Consequently, the end of the sleeping period was set arbitrarily at the time when the dog had regained sufficient coordination to walk on a polished floor.

One male and three female dogs were thyroidectomized, and permitted about four weeks' postoperative recovery. Intraperitoneal injections of 32.5 mg./Kg. sodium pentobarbital were then given weekly for seventeen consecutive weeks. The drug was then discontinued for several weeks, following which 65 mg. desiccated thyroid per day was administered orally. After one week of thyroid treatment, injections of pentobarbital were resumed for ten consecutive weeks, during which thyroid administration was continued.

Two normal female dogs were given 17 consecutive injections of pentobarbital at weekly intervals as described above. These animals were then thyroidectomized and permitted to recover about four weeks postoperatively. Pentobarbital injections were then given weekly for ten consecutive times.

RESULTS

Rats.—Table I summarizes the data obtained from the experimental and control rats. The thyroidectomized females show the greatest change, the average sleeping time in these being prolonged over that of the controls by twenty-one minutes. These data were treated statistically and the standard error of the difference between the two means determined by methods described by Davenport and Ekas (10). The difference between the means exceeded twice the standard error of the difference by about 17%, suggesting that a true difference existed in the duration of anesthesia between experi-

TABLE I.—DURATION OF PENTOBARBITAL ANESTHESIA IN RATS

Condition of Animals	Total No. of Rats		Total No. of Trials		Average Sleeping Time		Mean Difference		Standard Error of Difference	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Thyroidectomized	16	38	42	105	122	167	18	21	10.82	8.55
Controls	14	25	30	59	104	146	..	..	...	...
Thyroid fed	0	13	0	29	0	121	..	10.1	...	12.58
Controls	0	9	0	23	0	131	..	..	...	...
Thiouracil fed	0	29	0	83	0	154	..	14.0	...	9.67
Controls	0	9	0	29	0	140	..	..	...	...

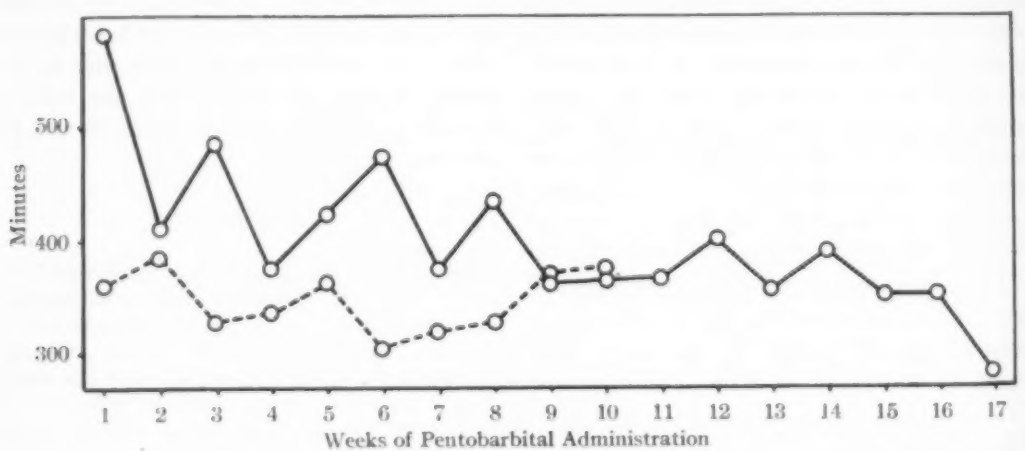


Fig. 1.—Solid line shows average sleeping time of 4 dogs after thyroidectomy. Dotted line shows sleeping time of same dogs during administration of 65 mg. thyroid per day.

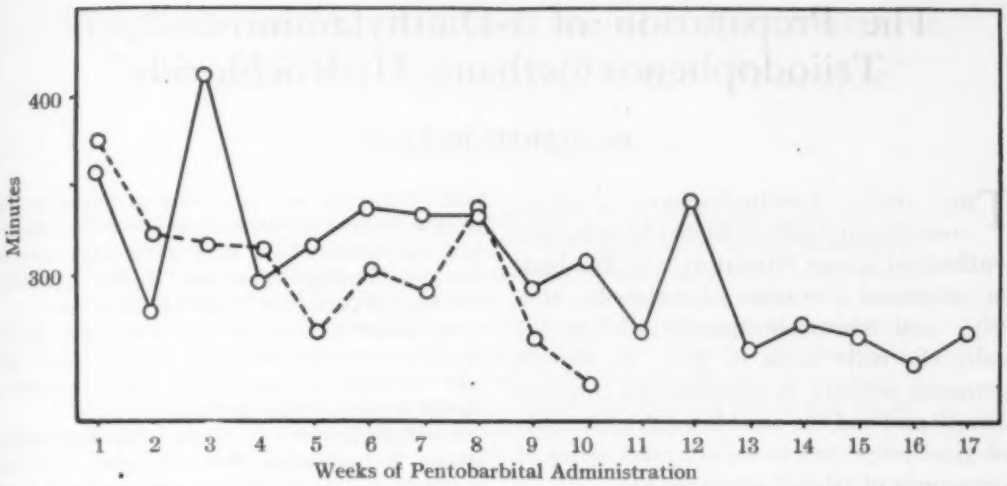


Fig. 2.—Solid line shows average sleeping time of 2 dogs before thyroidectomy. Dotted line shows sleeping time of same dogs postoperatively.

mentals and controls. The operated males likewise slept slightly longer than controls, but application of the statistical procedure above disclosed that no significant difference existed between means of the operated and control rats. Thyroid-fed females did not sleep quite as long as the controls, but here again statistical treatment failed to disclose a significant difference. The mean of thiouracil-fed females suggested that these slept longer than controls, but statistically this could not be established as a true difference (Table I).

Dogs.—The graph in Fig. 1 compares the sleeping time of four thyroidectomized dogs with that found in the same dogs during thyroid administration. Although a decrease in sleeping time appears to accompany thyroid feeding, this difference can be ascribed to barbiturate tolerance developed in the course of the experiment. This is indicated by the fact that the graph of sleeping time during thyroid administration continues at about the same level as the last few trials previous to hormone feeding. Ettinger (11) reported a similar tolerance to pentobarbital in dogs.

The graph in Fig. 2 shows the average duration of pentobarbital anesthesia in two normal dogs compared with that found in the same animals after thyroidectomy. No significant difference is indicated by these data.

DISCUSSION

Since the thyroid gland controls metabolism to a considerable degree, changes in its function could be expected to influence organs involved in the detoxification and excretion of pentobarbital. However, only in thyroidectomized female rats could a significant difference in the length of action of pentobarbital be demonstrated. Thyroidectomy, thiouracil feeding, and thyroid administration did not produce marked changes in the duration of pentobarbital anesthesia in the other experiments.

SUMMARY

1. Thyroidectomy in female rats tended to prolong the sleeping period resulting from sodium pentobarbital administration.

2. Thyroidectomy in male rats did not significantly alter the length of the sleeping time.

3. Administration of thyroid to rats did not significantly alter the period of sleep induced by pentobarbital.

4. Thiouracil feeding did not influence sleeping time to any significant degree.

5. In dogs, neither thyroidectomy nor thyroid administration to the operated dogs had any appreciable influence on the length of sleep induced by sodium pentobarbital.

REFERENCES

(1) Westfall, B. A., *J. Pharmacol. Exptl. Therap.*, **87**, 33(1946).
(2) Freireich, A. W., and Landsberg, J. W., *J. Am. Med. Assoc.*, **131**, 661(1946).
(3) Raventos, J., THIS JOURNAL, **64**, 355(1938).
(4) Gaylord, Charles, and Hodge, Harold C., *Proc. Soc. Exptl. Biol. Med.*, **55**, 46(1944).
(5) Furhman, F. A., *Science*, **105**, 387(1947).
(6) Rowbottom, Stanley, *Lancet*, **220**, No. 1, 439(1931).
(7) Scarborough, E. M., *J. Physiol.*, **86**, 183(1936).
(8) Warkentin, J., Warkentin, L., and Ivy, A. C., *Am. J. Physiol.*, **139**, 139(1943).
(9) Reineke, E. P., Mixner, J. P., and Turner, C. W., *Endocrinology*, **36**, 64(1945).
(10) Davenport, C. B., and Ekas, Merle P., "Statistical Methods in Biology, Medicine, and Psychology," ed. 4, John Wiley and Sons, New York, 1936, p. 37.
(11) Ettinger, G. H., *J. Pharmacol. Exptl. Therap.*, **63**, 82 (1938).

The Preparation of 1-Diethylamino-2-(2,4,6-Triiodophenoxy)ethane Hydrochloride*

By ALFRED BURGER

THE DRUG 1-diethylamino-2-(2,4,6-triiodophenoxy)ethane hydrochloride, first synthesized in our laboratory (1), has been the subject of a number of metabolic, toxicity, and chemotherapeutic studies (2) under the code name of B-9. It showed promising activity in experimental tuberculosis (2) and had the most favorable pharmacological properties in an extended series of compounds of related structure (3).

On several occasions the author has had to prepare considerable batches of the drug to supply physicians for preliminary clinical trials. Since a number of agencies have recently expressed interest in testing the drug it appears advisable to compile revised directions for its preparation, incorporating the experiences obtained in larger runs.

The success of the Williamson type condensation described below depends largely on the use of pure starting materials and the avoidance of overheating in the various phases of the reaction.

EXPERIMENTAL

β-Diethylaminoethyl Chloride Hydrochloride.—In a 2-L. three-necked flask equipped with a sealed mechanical stirrer, dropping funnel, and reflux condenser connected to a gas trap place a mixture of 200 Gm. of β-diethylaminoethanol and 300 cc. of dry benzene. Cool in a freezing mixture, and drop a solution of 262 Gm. of thionyl chloride in 200 cc. of dry benzene into the stirred mixture over a period of three to four hours. Remove the ice bath, place the flask on a steam bath, and heat the mixture to reflux for four hours, maintaining stirring as long as the accumulating thick mass of crystals will permit.

Cool the mixture, filter the hydrochloride with suction, wash it several times with cold acetone, and finally with a mixture of one part acetone and one part ether. Dry well in a vacuum desiccator and recrystallize the salt from about 400 cc. of ethanol. Continue recrystallizing until the melting point of the now nonhygroscopic salt reaches 207–208°. The yield of purest salt is 100 Gm. or better.

Additional fractions of lesser purity may be obtained by concentration of the mother liquors and used for other purposes. Only the best material should be used in the next step.

1 - Diethylamino - 2 - (2,4,6 - Triiodophenoxy)-ethane Hydrochloride (B-9).—Prepare a solution of 19.0 Gm. of clean sodium in 450 cc. of 99% methanol, and dissolve in it 190 Gm. of pure 2,4,6-triiodophenol. As soon as this compound has dissolved, add a solution of 70 Gm. of β-diethylaminoethyl chloride hydrochloride in 150–200 cc. of methanol with mechanical stirring. Sodium chloride precipitates immediately. Stir and reflux for two and one-half to three hours and stop heating at the first sign of definite darkening. Remove the solvent at 50° under 12–15 mm. pressure and treat the yellow, semisolid residue with alkaline water. Extract the base into several liberal portions of ether, combine the ether extracts, and wash them with several portions of 5% sodium hydroxide solution and with water until all iodinated sodium phenolates have been removed. Dry the ether solution over anhydrous sodium sulfate, filter, and evaporate the solvent at 35–40°, carefully avoiding overheating the residual pale yellow oil.

Dissolve the oily base in good acetone without delay, and neutralize with absolute ethanolic hydrogen chloride to Congo-blue reaction. The salt (B-9) crystallizes soon. If necessary, some ether may be added to induce crystallization.

Filter the drug, wash it with a mixture of acetone and ether, and recrystallize from methanol and a little acetone four to six times without prolonged warming. The last mother liquors must be colorless. Wash the salt with acetone and finally with ether. The colorless crystals melt at 196° (decomp.) without preceding sintering. The yield varies from 40–50%.

REFERENCES

(1) Long, L., and Burger, A., *J. Am. Chem. Soc.*, **63**, 1586 (1941).
(2) Saz, A. K., Johnston, F. R., Burger, A., and Bernheim, F., *Am. Rev. Tuberc.*, **48**, 40(1943).
(3) Burger, A., Wilson, E., Brindley, C. O., and Bernheim, F., *J. Am. Chem. Soc.*, **67**, 1416(1945).

* Received May 20, 1947, from the Cobb Chemical Laboratory, University of Virginia, Charlottesville, Va.

The Use of Acetylation in the Quantitative Separation of Ephedrine from Procaine*

By LLEWELLYN H. WELSH

Aqueous solutions of ephedrine give practically quantitative yields of the N-acetyl derivative by the action of acetic anhydride in the presence of excess bicarbonate. Under the same conditions procaine appears to acetylate at its primary amino group to yield a product which is basic by virtue of its tertiary nitrogen. The wide difference in properties of the acetyl derivatives of the two drugs permits their separation by simple extraction procedures, and the gravimetric determination of ephedrine as its acetyl derivative. The presence of small amounts of bisulfite greatly hinders the acetylation of ephedrine. The presence of ephedrine does not interfere in the determination of procaine by bromination.

RECENT WORK in this laboratory (1) established that the reaction of ephedrine with acetic anhydride at 70° yields the neutral N-acetyl derivative (hydroxyamide, I), m. p. 86–87°, and that the corresponding O-acetyl compound (aminoester, II) is capable of only a transitory existence before quantitatively rearranging into the hydroxyamide.

$C_6H_5CHOHCH(CH_3)N(CH_3)COCH_3$
(I)

$C_6H_5CH(OCOCH_3)CH(CH_3)NHCH_3$
(II)

These facts suggest the possibility of using acetylation in the analysis of drug mixtures containing certain aminoalcohols. If acetylation occurs quantitatively under conditions similar to those employed for the conversion of phenetedin to acetophenetedin (2), it should be possible to separate ephedrine and, perhaps, other aminoalcohols having an ArCHOHCNH grouping from compounds containing unacetylatable basic (e.g., tertiary amino) groups.

It was found that acetylation of ephedrine at room temperature occurs rapidly in aqueous solution containing excess bicarbonate, and yields 98.0–99.4 per cent of the theoretical amount of acetyl derivative. The acetylation was then carried out on samples containing 150 mg. of ephedrine hydrochloride and 30 mg. of procaine hydrochloride. After acidification of the reaction mixture, the acetylephedrine was separated from the acetylated procaine salt by extraction with chloroform, and was determined gravimetrically after removal of the solvent. Recoveries of 97.6–99.2 per cent were obtained. When the procedure was applied to a commercial product purporting to contain in each cc. 50 mg. of ephedrine hydrochloride, 10 mg. of procaine hydrochloride, and "not more than" 1.5 mg. of sodium bisulfite, the results indicated a shortage of 10–14 per cent of ephedrine salt. Total solids and chloride determinations, however, supported the label declaration. Accordingly, a solution was prepared which contained *ca.* 3 mg. of sodium metabisulfite per 100 mg. of ephedrine hydrochloride. When aliquots of the solution were subjected to the acetylation procedure, recoveries of approximately 53% resulted. The unacetylated ephedrine could be extracted with chloroform after rendering the aqueous phase alkaline with sodium hydroxide. If, however, before acetylating the bisulfite was destroyed by the addition of iodine-potassium iodide solution, normal recovery percentages of acetyl derivative were obtained. The reason for the interference of bisulfite in the acetylation of ephedrine is not apparent to the writer. The molecular proportions of bisulfite, ephedrine, acetic anhydride, and bicarbonate present in the acetylation of the prepared solution are approximately 1:32:450:1500, respectively. Whatever the interference mechanism, one molecule of bisulfite effectively

* Received June 27, 1947, from the Chemical Section, Medical Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.

blocks the acetylation of approximately fifteen molecules of ephedrine under the conditions employed.

Although procaine appears to acetylate at its primary amino group under the conditions used in the acetylation of ephedrine, the derivative obtained could not be induced to crystallize. Procaine was determined in the presence of ephedrine by extracting the two compounds from alkaline solution into chloroform, transferring them from this solvent into aqueous solution by shakeouts with dilute acid, adding a slight excess of standard bromide-bromate solution to the acid extract, and back-titrating with thiosulfate after adding potassium iodide. Under these conditions ephedrine did not interfere with the bromination even when present in amounts five times that of the procaine, and the bromination results were slightly better than 99 per cent of the theoretical. The determination of procaine by this method is considered to be expedient rather than ideal, since it gives no information concerning the nature of the material brominated.

EXPERIMENTAL

(Melting points are corrected.)

Acetylation Procedure.—Sodium bicarbonate (3.0 Gm.) was added to a separatory funnel containing *ca.* 150 mg. of ephedrine hydrochloride dissolved in 10 cc. of water. The funnel was swirled until the liquid was practically saturated with bicarbonate, and a total of 1 cc. of acetic anhydride was then added in three approximately equal portions. After each addition of anhydride the funnel was securely stoppered and shaken vigorously. From time to time the shaking was interrupted and the stopcock was opened to release internal pressure. A fresh portion of anhydride was not added until carbon dioxide evolution resulting from the previous addition had practically ceased, and after each reaction small amounts of fluid in the stem and on the stopper and mouth of the funnel were rinsed back into the main body of liquid with a few drops of water. The reaction mixture was allowed to stand fifteen minutes after effervescence resulting from the last addition of anhydride apparently had ceased. It was then made acid to Congo red with 10% (*w/v*) sulfuric acid (about 20 cc. was required), an additional 5 cc. was added, and the acetylephedrine was extracted immediately with five 15-cc. portions of chloroform. Each extract was shaken in another funnel with a solution of 0.5 Gm. of sodium bicarbonate in 6 cc. of water to remove acetic acid before filtering through a pledget of cotton into a beaker. The filtered extracts were concentrated to a small volume, transferred with chloroform to a tared 50-cc. beaker (previously dried at 105° and cooled in a desiccator), and the remainder of the solvent removed on the steam bath in a current of air. The residual resin of acetylephedrine was heated thirty minutes in an oven at 105°, cooled in a desiccator, and weighed rapidly. Although crystalline anhydrous acetylephedrine does not tend to gain weight in the air under ordinary conditions, the supercooled substance does appear to gain slightly possibly because of a tendency to form the monohydrate (3). The weight of anhydrous residue multiplied by 0.9731 represents the equivalent of ephedrine hydrochloride. Residues of acetylephedrine crystallized slowly when seeded and triturated. The substance is not appreciably volatile at 105°, since a 500-mg. sample suffered an apparent loss of only 0.5 mg. when left thirty minutes in an oven at this temperature.

When bisulfite was present it was removed prior to the introduction of bicarbonate by swirling the funnel and adding, dropwise, a solution of 0.5 Gm. of iodine and 1.5 Gm. of potassium iodide in 25 cc. of water. After a slight excess of iodine had been added, a few drops of starch solution were introduced, and the blue color was discharged by addition of the necessary amount of 0.1 *N* sodium thiosulfate.

Table I lists recoveries obtained when the acetylation was carried out on solutions containing ephedrine hydrochloride (150 mg.) only, and solutions containing, in addition, procaine hydrochloride (30 mg.) and sodium metabisulfite (*ca.* 4.5 mg.). Melting points of various acetylephedrine residues are also listed.

TABLE I

Compounds Added	% Recovery	M. P. of Acetylephedrine
None	99.4	84.5–86°
	98.0	
Procaine	98.6	
	97.7	
	99.2	85 –86°
	97.6	
Bisulfite	51.7[a]	84.5–86°
	53.7[a]	
	97.9[b]	
	98.8[b]	85 –86°
Procaine and bisulfite	49.8[a]	85 –86°
Av.	98.4	

[a] Not included in average.
[b] Bisulfite removed with iodine before acetylation.

After acetylephedrine had been extracted from one of the reaction mixtures containing bisulfite, the aqueous solution was rendered alkaline and extracted several times with chloroform. Addition of hydrogen chloride and subsequent evaporation of solvent yielded ephedrine hydrochloride, m. p.

217–218°, equivalent to 97.2% of the ephedrine which had escaped acetylation.

When the procedure (modified in that extraction was made from alkaline solution) was applied to a 150-mg. sample of procaine hydrochloride, a 100.3% recovery of acetylprocaine was obtained. The colorless, resinous material showed a neutralization equivalent of 285 (calcd. 278.3 for $C_{15}H_{22}N_2O_3$). Hazard and Corteggiani (4) have reported a melting point of 74° for acetylprocaine, but material prepared in this laboratory could not be obtained in a crystalline condition. After the extraction of acetylephedrine from acidified reaction mixtures also containing acetylprocaine, the latter substance was extracted into chloroform after basifying the aqueous phase with sodium hydroxide. The approximately 30-mg. residues of acetylprocaine obtained after solvent removal amounted to 90–103% of the calculated quantity.

Determination of Procaine.—One cubic centimeter of 20% sodium hydroxide was added to 10 cc. of a solution containing 100 mg. of procaine hydrochloride, 500 mg. of the corresponding ephedrine salt, and 15 mg. of sodium metabisulfite. The alkaline solution was extracted with six 10-cc. portions of chloroform. The combined chloroform extracts were shaken with two 10-cc. portions of 5% sulfuric acid, then with four 10-cc. portions of water. The acid solution of ephedrine and procaine sulfates was washed with 5 cc. of petroleum ether and drained into a flask. The petroleum ether was then washed three times with 10-cc. portions of water which were added to the contents of the flask. The acid solution was allowed to stand, with occasional shaking, until the odor of petroleum ether had practically disappeared and no fine droplets of the solvent could be observed. The volume was then adjusted to about 150 cc. by the addition of water and 9 cc. of conc. hydrochloric acid, and two drops of 0.1% methyl red indicator were added. The solution was brominated essentially as described by Wells (5). Tenth-normal bromide-bromate solution was added from a burette at a rate of approximately two drops per second while the contents of the flask were constantly agitated. The indicator added originally faded gradually during the bromination, and an additional one or two drops were necessary before the end of the titration. As the anticipated end point was approached the speed of addition of brominating reagent was reduced and the effect of one-drop additions was observed. The approximate end point was reached when the indicator suddenly became decolorized, and a faint odor of bromine was noticeable. About 1 Gm. of potassium iodide was then dissolved in the solution, and back-titration with 0.1 *N* sodium thiosulfate was effected in the presence of starch. The required thiosulfate varied between 0.1 and 0.2 cc. Results of determinations ranged from 99.2% to 99.4% of the theoretical. A blank determination carried through the several stages described above, but in the absence of procaine, resulted in the consumption of only 0.03 cc. of bromide-bromate solution.

SUMMARY

Aqueous solutions of ephedrine give practically quantitative yields of the N-acetyl derivative by the action of acetic anhydride in the presence of excess bicarbonate. Under the same conditions procaine appears to acetylate at its primary amino group to yield a product which is basic by virtue of its tertiary nitrogen. The wide difference in properties of the acetyl derivatives of the two drugs permits their separation by simple extraction procedures, and the gravimetric determination of ephedrine as its acetyl derivative. The presence of small amounts of bisulfite greatly hinders the acetylation of ephedrine.

The presence of ephedrine does not interfere in the determination of procaine by bromination.

REFERENCES

(1) Welsh, L. H., *J. Am. Chem. Soc.*, **69**, 128(1947).
(2) Emery, W. O., *U. S. Dept. Agr. Bur. Chem. Bull.*, No. 162, 193(1913).
(3) Nagai, N., *J. Pharm. Soc. Japan*, No. 127, 832(1892); Mitchell, W., *J. Chem. Soc.*, **1940**, 1153.
(4) Hazard, R., and Corteggiani, E., *Compt. rend.*, **216**, 779(1943).
(5) Wells, E., *J. Assoc. Official Agr. Chem.*, **25**, 537(1942)

The Synthesis and Study of Some N-Dihydroxypropyl Derivatives*

By JAMES W. JONES† and ALFRED HALPERN‡§

The N-dihydroxypropyl derivatives of sulfathiazole, saccharin, and phenobarbital have been prepared by the reaction between both the sodium and the silver salts of the imides with glyceryl-α-chlorhydrin. Attempts at crystallization from various solvents failed to yield crystalline material. The influence of silver hydroxide and copper powder on the yield of product from the above reaction was determined The solubilities of the dihydroxypropyl derivatives in water were studied.

RECENTLY it was shown that the introduction of the dihydroxypropyl group in theophylline did not change the therapeutic properties of the compound and led to a stable, water-soluble derivative (1). It became of interest, therefore, to determine the effect on the solubility by the introduction of the dihydroxypropyl group in other nitrogen compounds difficulty soluble in water.

Several compounds of therapeutic importance, of which an acid-stable water-soluble derivative would be advantageous, were chosen for the study. Sulfathiazole (a representative "sulfa" drug), saccharin, and phenobarbital (a typical barbiturate) were the imides selected for N-dihydroxypropyl substitution. These compounds are all soluble in dilute alkali. However, their alkali salts are probably decomposed to liberate the water-insoluble base in the gastric fluids as was shown for the sodium salt of theophylline (1).

In the case of the "sulfa" drugs a stab e water-soluble derivative would prevent crystallization in the urinary tract. A soluble saccharin derivative would be of value in preparing aqueous pharmaceuticals of acid reaction. A neutral, water-soluble barbiturate would greatly enhance the parenteral administration of these drugs.

The N-hydroxyethyl derivative of phenobarbital has been prepared by the reaction between the sodium salt of phenobarbital and ethylenemonochlorhydrin (2). A huge excess of the ethylenemonochlorhydrin was used and the N-derivative was obtained in fairly good yields. A modification of this method was used as a general method for the preparation of the N-dihydroxypropyl derivatives.

EXPERIMENTAL

Method A.—To 0.1 mole of the sodium salt of the imide, in a 3-neck flask fitted with a stirrer and a thermometer, was added 1.5 mole of glyceryl-α-chlorhydrin. The mixture was heated on an oil bath for two hours at a temperature of 120–130°. Rapid stirring was maintained throughout the reaction period. At the end of this time, the reaction mixture was cooled, and the stirring stopped. The mixture was filtered by suction and the residue on the filter paper washed with two 20-cc. portions of hot absolute ethanol. The residue on the filter paper, which was identified as sodium chloride, weighed 5.2 Gm. The theoretical quantity was 5.8 Gm. The excess glyceryl-α-chlorhydrin and the ethanol were removed by distillation under reduced pressure. The residue was a brown, gummy material.

The residues were purified through repeated precipitation by ether from absolute ethanol. After four such precipitations, a colorless oil was obtained, which, after the removal of the last traces of the solvent under reduced pressure, hardened to a plastic semisolid resin. The yield of purified derivative was:

	Yield		
	Found, Gm.	Theoretical, Gm.	%
(I) N-Dihydroxypropyl-sulfathiazole	20.4	31.4	65
(II) N-Dihydroxypropyl-phenobarbital	19.8	29.1	68
(III) N-Dihydroxypropyl-saccharin	16.5	24.2	68

% Nitrogen (Kjeldahl)
Calc'd.: (I) 13.37, (II) 9.62, (III) 7.64
Found : (I) 13.47, (II) 9.71, (III) 7.41

Method B.—One-tenth mole of the sodium salt of the imide was dissolved in 300 cc. of distilled water and heated to boiling. Five-tenths mole of glyceryl-α-chlorhydrin was added and the solution boiled for two hours, water being added from time

* Received May 19, 1947, from the State University of Iowa, College of Pharmacy, Iowa City, Iowa.
† Professor of Pharmacy, State University of Iowa.
‡ Graduate Student, State University of Iowa and Fellow of the American Foundation for Pharmaceutical Education.
§ Present address: Duquesne Univ., College of Pharmacy.

to time to maintain the volume. The water was then removed under reduced pressure and the residue extracted with five 50-cc. portions of hot absolute ethanol. The sodium chloride remaining weighed 4.8 Gm. The ethanol was removed by distillation under reduced pressure and the residue purified in the above manner. The products of these reactions were also plastic semisolid materials. The yield of product obtained:

	Yield		
	Found, Gm.	Theoretical, Gm.	%
(I) N-Dihydroxypropyl-sulfathiazole	12.5	31.4	40
(II) N-Dihydroxypropyl-phenobarbital	12.2	29.1	42
(III) N-Dihydroxypropyl-saccharin	9.9	24.2	41

% Nitrogen (Kjeldahl)
Calc'd.: (I) 13.37, (II) 9.62, (III) 7.64
Found : (I) 13.26, (II) 9.42, (III) 7.40

Method C.—Substituting the silver salt for the sodium salt in both reactions (A and B) resulted in products identical to those obtained from the sodium salts. A quantitative yield of silver chloride was obtained. The yield of N-dihydroxypropyl imide obtained from the silver salt in Method A was:

	Yield		
	Found, Gm.	Theoretical, Gm.	%
(I) N-Dihydroxypropyl-sulfathiazole	25.7	31.4	82
(II) N-Dihydroxypropyl-phenobarbital	24.3	29.1	84
(III) N-Dihydroxypropyl-saccharin	20.0	24.2	83

% Nitrogen (Kjeldahl)
Calc'd.: (I) 13.37, (II) 9.62, (III) 7.64
Found: (I) 13.41, (II) 9.60, (III) 7.58

The yield of N-dihydroxypropyl imide obtained from the silver salt in Method B was:

	Yield		
	Found, Gm.	Theoretical, Gm.	%
(I) N-Dihydroxypropyl-sulfathiazole	23.8	31.4	76
(II) N-Dihydroxypropyl-phenobarbital	23.2	29.1	80
(III) N-Dihydroxypropyl-saccharin	18.1	24.2	79

% Nitrogen (Kjeldahl)
Calc'd.: (I) 13.37, (II) 9.62, (III) 7.64
Found: (I) 13.40, (II) 9.56, (III) 7.54

Preparation of the Silver Salt of the Imides (3).—One-tenth mole of the sodium salt of the imide was dissolved in 300 cc. of distilled water. The solution was stirred with a mechanical stirrer and a solution of 0.1 mole of silver nitrate in 200 cc. of distilled water was added. An immediate precipitation occurred and the mixture was stirred for fifteen minutes longer. The precipitate was filtered off on a Büchner funnel and washed well with distilled water, then dried in a vacuum desiccator overnight. The silver salts must be protected from light to prevent darkening of the compound. The yield of silver imide obtained was:

	Yield		
	Found, Gm.	Theoretical, Gm.	%
Silver sulfathiazole	33.5	36.3	90
Silver phenobarbital	36.7	39.9	92
Silver saccharin	27.6	29.0	95

Method D.—Reactions A and B were repeated with the addition of silver hydroxide and copper powder as catalysts. One-half gram of freshly precipitated AgOH was added to the reaction mixture and the process carried out in the indicated manner. The average yield of substituted imide was increased. The yield of compound obtained from Method A catalyzed by silver hydroxide was:

	Yield		
	Found, Gm.	Theoretical, Gm.	%
N-Dihydroxypropyl-sulfathiazole	23.1	31.4	74
N-Dihydroxypropyl-phenobarbital	22.9	29.1	79
N-Dihydroxypropyl-saccharin	18.8	24.2	78

The yield of compound obtained from Method B catalyzed by silver hydroxide was:

	Yield		
	Found, Gm.	Theoretical, Gm.	%
N-Dihydroxypropyl-sulfathiazole	22.2	31.4	71
N-Dihydroxypropyl-phenobarbital	21.1	29.1	73
N-Dihydroxypropyl-saccharin	17.4	24.2	72

Copper powder was used in a similar manner and the yields in this instance were increased, though not as high as with silver hydroxide. The yield of compound obtained from Method A catalyzed by copper powder was:

	Yield		
	Found, Gm.	Theoretical, Gm.	%
N-Dihydroxypropyl-sulfathiazole	21.6	31.4	69
N-Dihydroxypropyl-barbital	20.9	29.1	72
N-Dihydroxypropyl-saccharin	16.9	24.2	74

The yield of compound obtained from Method B catalyzed by copper powder was:

	Yield		
	Found, Gm.	Theoretical, Gm.	%
N-Dihydroxypropyl-sulfathiazole	20.0	31.4	64
N-Dihydroxypropyl-phenobarbital	19.1	29.1	66
N-Dihydroxypropyl-saccharin	16.2	24.2	67

N-Allylphenobarbital was prepared using 0.1 molar quantities from ethyl phenyl-ethyl malonic ester and allyl urea in the presence of sodium methylate and methyl alcohol. The yield was 63% of the theoretical. Melting point found: 67–68°. Recorded melting point: 68–69° (4).

The dihydroxypropyl derivative of phenobarbital was obtained by the oxidation of N-allylphenobarbital with cold, dilute, aqueous potassium permanganate. A product identical to that obtained by the previous reactions was obtained. The yield was 52% of the theoretical. Nitrogen calculated: 9.62. Nitrogen found: 9.31.

Attempts at crystallizing the dihydroxy derivatives from acetone, acetone-benzene, benzene, benzene-ligroin, alcohol, aqueous alcohol, alcohol-ether, and water solutions failed. The product obtained in all cases was a plastic, semisolid resin.

It was of interest to note that hydroxymethylene-bis(1-methylenephenobarbital) prepared by the reaction between glyceryl α-γ dibromohydrin and phenobarbital in the presence of sodium in methanol was reported as a hard glassy solid without definite melting point (2). Furthermore, the gum obtained from this reaction could not be induced to crystallize from any of the usual organic solvents.

The substituted compounds are stable at room temperature. They decompose on heating above 180°. Attempts at distillation of the compounds at 2 mm. Hg pressure resulted in decomposition of the products.

The N-dihydroxypropyl derivatives all gave a positive periodic acid test (5) for the glycol group.

The compounds were soluble in alcohol, slightly soluble in acetone, and insoluble in ether, chloroform, and petroleum ether. The solubility of the compounds in water at 25° was found to be as follows:

N-Dihydroxypropyl-sulfathiazole	1 Gm. in 330 cc.
N-Dihydroxypropyl-phenobarbital	1 Gm. in 200 cc.
N-Dihydroxypropyl-saccharin	1 Gm. in 90 cc.

The introduction of the N-dihydroxypropyl or N-ethylhydroxy group in saccharin resulted in a tasteless compound.

The solubility of the compounds in water was increased by the introduction of the dihydroxypropyl group. The noncrystalline nature of the compounds, however, greatly diminished the advantages of solubility thus obtained. The rather lengthy purification procedures necessary to obtain a pure compound also detracted from the increased solubility advantage. Further study of these compounds was discontinued for the above reasons.

SUMMARY AND CONCLUSIONS

1. The effect on solubility in water by the substitution of the dihydroxypropyl group in difficultly soluble imides was studied.

2. The N-dihydroxypropyl derivatives of sulfathiazole, saccharin, and phenobarbital were prepared by the reaction between the sodium salt of the imide and glyceryl-α-chlorhydrin. The compounds were also prepared from the silver salts of the imide and glyceryl-α-chlorhydrin.

3 The products were all plastic, semisolid, resinous materials. Attempts at crystallization from various solvents failed to yield a crystalline material.

4. The influence of silver hydroxide and copper powder on the yield of product from the above reaction was noted.

5. N - Dihydroxypropylphenobarbital prepared by the oxidation of N-allylphenobarbital with potassium permanganate was identical to that obtained by the previous means.

6. The solubility of the dihydroxypropyl derivatives in water was determined.

7. The study was discontinued because the difficulties in obtaining a pure compound and the plastic semisolid resinous state detracted from the pharmaceutical utility of the crystalline parent compounds. The advantages of increased solubility did not compensate for the above disadvantages.

REFERENCES

(1) Maney, P. V., Jones, J. W., Gross, E. G., and Korns, H. M., THIS JOURNAL, **35**, 266–72(1946).
(2) Henze, H. R., and Spurlock, J. J., *J. Am. Chem. Soc.*, **63**, 3360(1941).
(3) Braun, C. E., and Towle, J. L., *ibid.*, p. 3523.
(4) Hoffman-LaRoche and Company, United Kingdom Patent No. 391,741, May 4, 1933.
(5) Shriner, R. L., and Fuson, R. C., "Systematic Identification of Organic Compounds," ed. 2, John Wiley and Sons, New York, 1940, p. 56.

Chemical Determination of Synthetic Estrogens
I. Basic Data on the Nitrosophenol Reaction*

By SIDNEY GOTTLIEB

A method is described for the identification and quantitative determination of diethylstilbestrol, hexestrol, dienestrol, benzestrol, and Meprane which depends on the formation of the corresponding o-nitrosophenols. The absorption of the alkaline quinoid forms of these derivatives in solution is a linear function of the concentration.

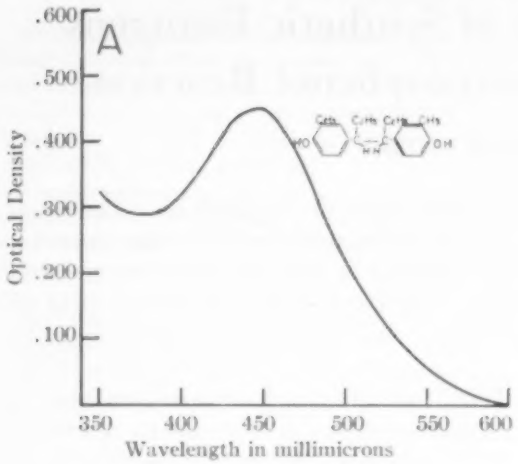
Dodds, *et al.* (1), reported the extremely high estrogenic activity of the 4,4′-stilbenediols in 1938. Since then this group of compounds has come into wide and important use in the treatment of various disorders stemming from estrogen deficiency. Virtually each year sees a new derivative of this series put on the market. A reliable and widely applicable method for the determination of these compounds is needed; it is the purpose of this contribution to propose such a method and to present the basic data on it.

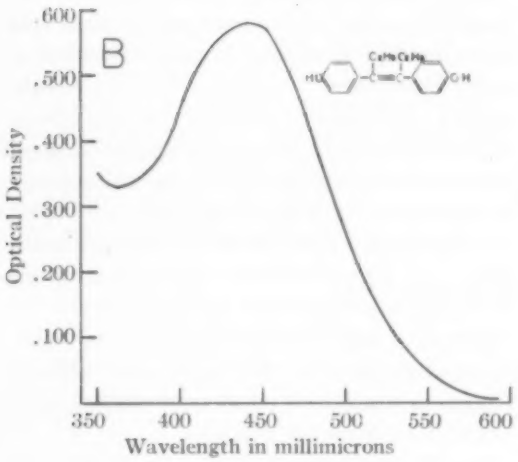
Various methods have been proposed for the chemical determination of compounds in the 4,4′-stilbenediol series. Elvidge (2) and Goetz and Seif (3) proposed absorption spectrophotometry of the unaltered compounds for diethylstilbestrol, diethylstilbestrol dipropionate, and hexestrol. Dingemanse (4) determined diethylstilbestrol by its reaction with antimony pentachloride to form a red addition compound. The color was reported to be relatively unstable and fats, sterols, and colored substances interferred with the reaction. Tubis and Bloom (5) suggested the use of the Folin and Ciocalteu reagent (molybdo-phosphotungstic acids) for the determination of diethylstilbestrol in tablets. This method has been included in the U. S. P. XIII for diethylstilbestrol capsule, injection, and tablet preparations. Since the reaction depends on the reduction of the labile phosphomolybdic-phosphotungstic acids to the blue oxides of molybdenum and tungsten, its use is limited in many types of preparations. The coupling of diethylstilbestrol with diazobenzenesulfonic acid to form a colored solution has been used by Huf and Widmann (6) as an assay for this estrogen. An accuracy of 10 per cent is claimed for the method and the diazotization to produce the reagent must be carried out daily. A bromometric procedure for determining 1–40 mg. of diethylstilbestrol has been described by Sondern and Burson (7) and the reaction of various stilbenediols with bromine in acetic acid has been used by Cocking (8) as a colorimetric assay procedure. Malpress (9) has elaborated a method depending on the nitration of diethylstilbestrol, hexestrol, and dienestrol to polynitro compounds and the subsequent measurement of the orange color produced by these nitro derivatives in alkaline solution. A determination of dienestrol in biological samples has been proposed by Smith (10) utilizing the formation and subsequent titration of the maleic acid adduct. Recoveries of 60–80 per cent of added dienestrol from urine are reported.

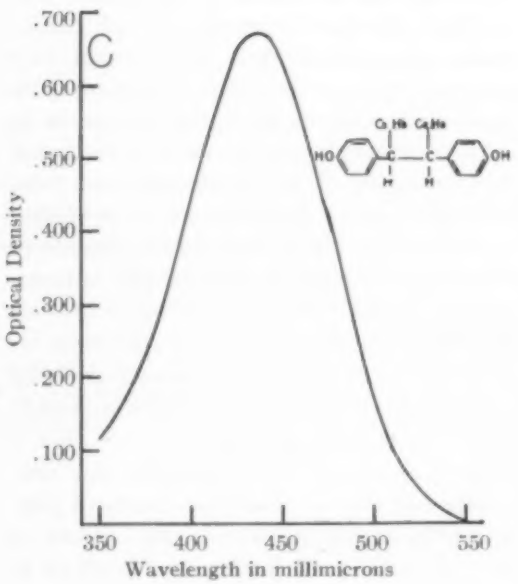
In this paper the author reports a method depending on the formation of nitrosophenols from various synthetic estrogens. The method affords an opportunity to distinguish among individual members of this group of compounds and to determine each compound quantitatively. In a review of color tests for phenols, Gibbs (15) discussed the formation of nitrosophenols and their quinone oximes. Stoughton (11) published a method for the estimation of phenols in biological materials which consisted of treating the phenol with nitric and sulfuric acids at 100° to form a nitrosophenol which rearranges in the presence of excess alcoholic ammonium hydroxide to form a highly colored quinoid compound.

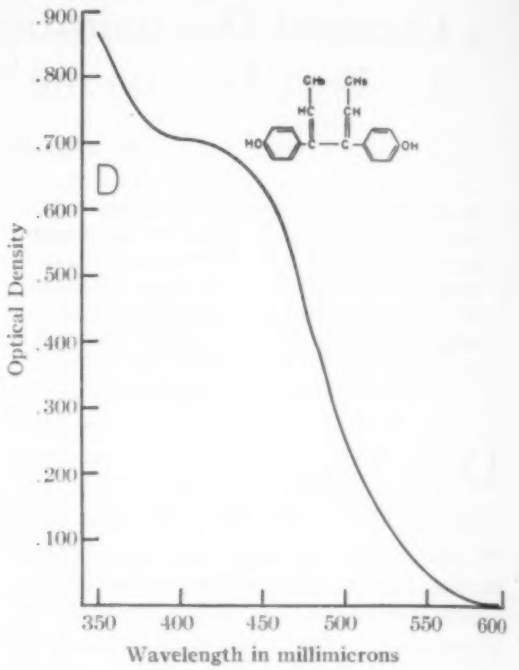
Wetlaufer, *et al.* (12) modified this procedure slightly to determine traces of phenols in hydrocarbon solvents and Lykken, *et al.* (13) made some further modifications in

* Received July 24, 1947, from the Chemical Section, Medical Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.









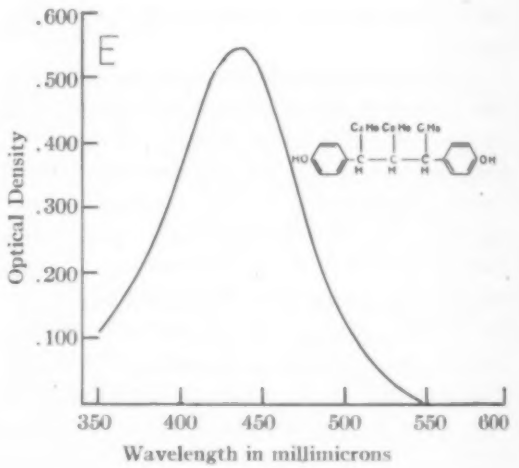
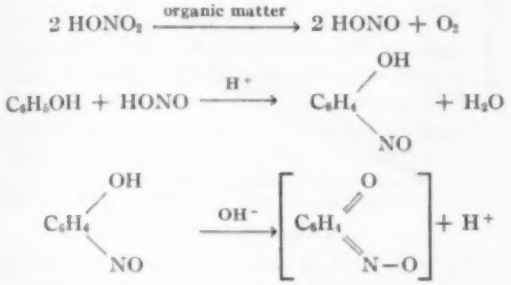


Figure 1 (A – E)

Absorption curves for nitrosophenol quinoid compounds from five synthetic estrogens.

A—phenol from Meprane
B—diethylstilbestrol
C—hexestrol
D—dienestrol
E—benzestrol

using the reaction for the same purpose. Using phenol as an example, the probable reactions are as follows:



The method described below has been applied to derive data on absorption and calibration curves for five important synthetic estrogens, namely diethylstilbestrol (3,4-bis - (*p* - hydroxyphenyl) - hexene - 3), hexestrol (3,4-bis-(*p*-hydroxyphenyl) hexane), dienestrol (3,4-bis-(*p*-hydroxyphenyl) hexadiene-2,4), benzestrol (2,4-bis-(*p*-hydroxyphenyl)-3-ethylhexane), and Meprane (3,4-bis-(*m*-methyl-*p*-propionoxyphenyl)-hexane). A method for the application of this reaction to pharmaceutical preparations has been worked out and is at present being collaboratively checked by several laboratories. It will be the subject of a subsequent publication.

EXPERIMENTAL

Reagents.—Buffer solution: To 800 cc. of glacial acetic acid, add 150 cc. of 10% potassium hydroxide solution and 50 cc. of water, and mix well.

Saturated aqueous sodium nitrite solution.

Concentrated sulfuric acid (36 *N*).

Alcoholic ammonium hydroxide solution. Mix 450 cc. anhydrous ethyl alcohol or isopropyl alcohol, 300 cc. of conc. ammonium hydroxide (14 *N*), and 250 cc. of water.

Method.—Make up a standard solution of the compound being investigated by dissolving 10 mg. in 100 cc. of 95% ethyl alcohol. For diethylstilbestrol, hexestrol, dienestrol, and benzestrol, make up a series of concentrations in the range of 0.00 to 1.00 mg. by evaporating the requisite amount of alcoholic solution in 100-cc. beakers. In the case of the phenol from Meprane, the range should be from 0.0 to 2.0 mg. The residue in each beaker is dissolved in 5 cc. of buffer solution; gentle warming may be necessary to completely dissolve several of the compounds. Five drops of concentrated sulfuric acid is then added, followed by 2 drops of saturated sodium nitrite solution. The solution is allowed to stand at room temperature for thirty minutes with occasional shaking. The solution is then transferred quantitatively to a 50-cc. volumetric flask and diluted to near the mark with alcoholic ammonium hydroxide solution, cooling the flask in an ice bath as the alkali is added, and using the alkali to wash out the beaker. Let stand thirty minutes, dilute exactly to the mark with alcoholic ammonium hydroxide solution, and filter through a dry filter. This filtered solution is then used to make the photometric measurements. The color is stable for at least several hours after filtration.

Hydrolysis of Meprane.—The free phenol was obtained in quantitative yield from its dipropionic acid ester by refluxing about 0.5 Gm. of the ester in a 10% methanolic solution of potassium hydroxide for one hour. The methanolic solution was cooled, diluted with 100 cc. of distilled water, acidified with 10% HCl, and quantitatively transferred to a separatory funnel. This solution was then extracted four times with 50-cc. portions of peroxide-free diethyl ether. The combined ether extracts were washed three times with 100-cc. portions of 5% sodium bicarbonate and twice with 100-cc. portions of water. The ether solution was then separated, filtered through a dry filter, the filter washed with ether and the ether solution evaporated to dryness. The phenol (3,4-bis-(*m*-methyl-*p*-hydroxyphenyl)-hexane) melted at 142–145° (corr.). Ten milligrams of this material was dissolved in 100 cc. of 95% ethyl alcohol to make up the standard solution.

Photometric Measurements.—The absorption curves in Fig. 1 were obtained by treating 1 mg. of each compound as described above under "Method," and studying the absorption of the solutions so obtained in the spectral range of 350 to 600 mμ. The instrument used was a Beckmann Model DU Quartz Spectrophotometer, with the blue-sensitive phototube in position. The standard calibration curves shown in Fig. 2 were obtained by measuring the absorption of each sample at a wave length of 440 mμ on the Beckmann instrument. For both the absorption and calibration curves, the instrument was set at 100% transmission (or zero optical density) for a tube containing a blank solution which had been run through the entire procedure, omitting the addition of any phenolic compound.

Stability of Color.—Lykken, *et al.* (13), report that the colors produced by nitrosophenol quinoid compounds slowly increase in intensity, and these investigators recommend allowing the solutions to stand overnight before measuring the color. The synthetic estrogens apparently do not show this instability when treated as described above. Table I portrays a typical experiment with diethylstilbestrol, indicating no appreciable fading over a period of four hours. The other estrogens behaved in a similar manner.

TABLE I.—STABILITY OF SOLUTIONS OF THE NITROSOPHENOL QUINOID FROM DIETHYLSTILBESTROL. VARIOUS AMOUNTS OF DIETHYLSTILBESTROL TREATED AS DESCRIBED UNDER "METHOD"

Diethylstilbestrol	Optical Densities of Solutions at —Various Times after Development of Color—				
Mg.	15 Min.	30 Min.	1 Hr.	2 Hr.	4 Hr.
0.1	0.051	0.060	0.062	0.060	0.061
0.5	0.280	0.295	0.293	0.295	0.294
1.0	0.509	0.580	0.580	0.575	0.579

DISCUSSION

It can be seen from Fig. 1 that the absorption curves of the quinoid compounds formed by the estrogens studied are sufficiently different to enable their identification in a preparation containing only one estrogen. The accurate quantitative determination of one in the presence of another would be possible only in the case of a hexestrol-dienestrol or a benzestrol-dienestrol mixture, but the simultaneous determination of other pairs might be possible if a study of the absorption of the quinoid compounds in the region below 350 mμ was made. At any rate, it is improbable that mixtures of these compounds would occur in pharmaceutical preparations.

The natural estrogens, estrone, and α-estradiol have been submitted to this procedure and their absorption at 440 mμ (Table II) was found to be so low in comparison to the synthetic estrogens that their contribution to the absorption in mixtures of synthetic and natural estrogens can be ignored, or, in very careful work, easily corrected for by a separate determination (14).

TABLE II.—OPTICAL DENSITY OF SOLUTIONS OF THE NITROSOPHENOL QUINOIDS FROM ESTRONE, α-ESTRADIOL, AND DIETHYLSTILBESTROL. ONE MILLIGRAM OF EACH COMPOUND TREATED AS DESCRIBED UNDER "METHOD"

Compound	Optical Density
Estrone	0.020
α-Estradiol	0.044
Diethylstilbestrol	0.580

It will be noticed that in the case of the phenol 3,4-bis(*m*-methyl-*p*-hydroxyphenyl)-hexane, the optical density is appreciably less than that of the other estrogens in comparable concentrations. This is undoubtedly due to the hindering effect of the methyl group in the position ortho to the phenolic group. The presence of these methyl groups probably effectively blocks two of the four possible sites for the introduction of the nitroso group in the molecule.

Although this method is specific for phenolic compounds, it will not distinguish among several phenolic compounds, unless the spectral characteristics of the quinoid compounds formed had been previously studied. However, as mentioned above, other phenols are usually not encountered in pharmaceutical preparations containing these estrogens.

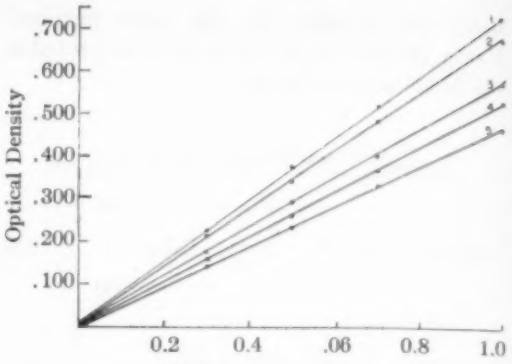


Fig. 2.—Standard calibration curves for the nitrosophenol quinoid compounds from five synthetic estrogens. 1—dienestrol; 2—hexestrol; 3—diethylstilbestrol; 4—benzestrol; 5—phenol from meprane.

SUMMARY

Basic data are presented on a new chemical method for the determination of the synthetic estrogenic compounds, 3,4-bis-(*p*-hydroxyphenyl)-hexene-3, 3,4-bis-(*p*-hydroxyphenyl)-hexane, 3,4-bis-(*p*-hydroxyphenyl)-hexadiene-2,4, 2,4-bis-(*p*-hydroxyphenyl)-3-ethylhexane, and 3,4-bis-(*m*-methyl-*p*-propionoxyphenyl)-hexane. A study of the absorption curves of the alkaline quinoid form of the ortho-nitrosophenols derived from these estrogens by a simple procedure indicates that they can be qualitatively identified by this means. Calibration curves are presented to indicate the correspondence of concentration and color in this reaction. The natural estrogens, estrone, and α-estradiol, do not interfere.

REFERENCES

(1) Dodds, E. C., Goldberg, L., Lawson, W., and Robinson, R., *Nature*, **141**, 247(1938).
(2) Elvidge, W. F., *Quart. J. Pharm. Pharmacol.*, **12**, 347 (1939).
(3) Goetz, H., and Seif, L. D., THIS JOURNAL, **34**, 209 (1945).
(4) Dingemanse, E., *Acta Brevia Neerland. Physiol., Pharmacol., Microbiol.*, **10**, 118(1940).
(5) Tubis, M., and Bloom, A., *Ind. Eng. Chem., Anal. Ed.*, **14**, 309(1942).
(6) Huf, E., and Widmann, G., *Z. physiol. Chem.*, **274**, 88(1942).
(7) Sondern, C. W., and Burson, C., *Ind. Eng. Chem., Anal. Ed.*, **14**, 358(1942).
(8) Cocking, T. T., *Analyst*, **68**, 144(1943).
(9) Malpress, F. H., *Biochem. J.*, **39**, 95(1945).
(10) Smith, A. E. W., *Nature*, **157**, 697(1946).
(11) Stoughton, R. W., *J. Biol. Chem.*, **115**, 293(1936).
(12) Wetlaufer, L. A., VanNatta, F. J., and Quattlebaun, H. B., *Ind. Eng. Chem., Anal. Ed.*, **11**, 438(1939).
(13) Lykken, L., Treseder, R. S., and Zahn, V., *ibid.*, **18**, 103(1946).
(14) Carol, J., and Rotondaro, F. A., THIS JOURNAL, **35**, 176(1946).
(15) Gibbs, H. D., *J. Biol. Chem.*, **71**, 445(1927).

Comparative *In Vitro* Neutralization of Heparin*

By E. J. MACKOWIAK and R. D. BARNARD

Four classes of antiheparinics have been examined for the *in vitro* restoration of spontaneous coagulability to heparinized human blood. Of the four antiheparinics studied, toluidine blue was found to be more effective than modified human globin, hematoxylin, or lactose.

THE HISTONE, protamine, has been used to neutralize the anticoagulant effect of previously administered heparin (1–3). Occasion for such use has seldom arisen and suitable pharmaceutical protamine preparations are consequently sparse. A broader significance of antiheparinic therapy was recently uncovered when it was discovered that bleeding in a certain category of hemorrhagic dyscrasias was due to an autochthonous increase of heparin in the blood. Volkert and Hertel (4) found heparin in identifiable and determinable quantities in the blood of acute leukemics and Barnard and Sprinz (5), in their series of acute leukemic patients, found that 40 per cent of deaths were due to heparinemic hemorrhage into a vital organ. It was furthermore found that the heparinemia in this condition, as in the hemorrhagic phases of thrombopenia, aplastic anemia, agranulocytosis, anaphylaxis, and radiation sickness was preceded by a failure of erythroid marrow function and a reduction in blood cholinesterase (6). This sequence—the atopic-exudative syndrome (7)—is rather more common in clinical medicine than is usually appreciated. Barnard and Sprinz (5) were able to restore the coagulability in the blood of a patient with terminal acute leukemia by the addition of the histone, modified human globin.

The emergence of antiheparinic therapy from its present role as an occasional procedure in correcting the effects of an overadministration of one medicinal agent with another, to that of its potentially greater role as a life-saving procedure in a wide category of hemorrhagic dyscrasias and the possible broadening of the scope of such therapy should radiation sickness develop into an industrial or a wartime hazard, led us to undertake a search for additional antiheparinics. Four classes of substances with antiheparinic potency have been investigated and will be discussed from a standpoint of possible therapeutic applicability. This discussion is based on the *in vitro* measurement of antiheparinic potency; the order of discussion is the chronology of investigation.

I. Modified Human Globin.—The material used by us was that developed as an oncotically active colloid by Dr. M. M. Strumia and his co-workers at the Bryn Mawr Hospital in Pennsylvania. Samples which had proved to be pyrogenic on human administration as well as nonpyrogenic samples proved to be equally effective in restoring spontaneous coagulability to heparinized human blood. The action is rather slow, about twelve hours being required for complete heparin neutralization regardless of the initial concentration of the latter. The presence of 400 mg. % of globin is necessary to vitiate 2 mg. % of heparin. Such a concentration of globin is readily attained clinically and 2 mg. % of heparin is the highest concentration that has been encountered in any instance of estrapenic shock. The slowness of action of globin, however, would seem to pose a theoretic disadvantage which might be offset by its relatively low toxicity and the future ready availability of the material with the re-advent of a National Blood Donor Program.

II. Toluidine Blue.—Allen and Jacobson (8) used toluidine blue to restore coagulability to the blood of dogs in the hemorrhagic phase of radiation sickness and to humans with acute leukemia. Neutralization of heparin by toluidine blue has long been known (9); in fact this dye had been used to trace the origin of heparin to the basophilic granules of the mast cells of Ehrlich (10).

Our experiments fully confirmed the findings of Allen and Jacobson. Whereas heparinemia is not the sole determining factor in bleeding during an atopic-exudative reaction, neutralization of the heparinemia serves to arrest such bleeding even though the cholinergic intoxication of the atopic-exudative syndrome persists. It was found that toluidine blue would almost instantly neutralize heparin and restore coagulability to fluid blood containing as much as 100 mg. % of heparin Toluidine blue is unquestionably the best antiheparinic (at least in its theoretic promise) that we have tested.

* Received August 12, 1947, from the Veterans Administration Hospital, Batavia, N. Y., and the Terrace Heights Hospital, Hollis, L. I., N. Y.

III. Hematoxylin.—Because hematoxylin also stains the basophilic granules in the mast cells and because it has proved nontoxic on oral administration (it has been used in doses as high as 5 Gm. as an astringent in diarrheas) the antiheparinic effect of this dye was tested *in vitro*. For concentrations up to 2% of heparin, it is equal in effectiveness to toluidine blue but for higher concentrations of the anticoagulant, the action of hematoxylin becomes complicated by a marked precipitation of plasma proteins.[1]

Whether the facility of oral administration and the cost of purification of logwood extracts will justify their exploitation as antiheparinics is a question that still remains to be decided.

IV. Polysaccharides.—Lactose, in solid phase, induces rapid coagulation of heparinized blood. Starch and glycogen have a similar, though weaker, effect. Solutions of polysaccharides containing no excess in solid phase are devoid of antiheparinic action. Presumably in this instance we are dealing with a surface reaction, the heparin being adsorbed by and its acid groups neutralized by the hydroxyls of the polysaccharide. There appears to be no chance for the pharmaceutic utilization of the heparin neutralizing properties of saccharides, at least in systemic antiheparinic therapy, and the phenomenon is included in the report only because of its possible academic interest.

[1] In this connection it must be pointed out that all electropositive colloid substances may precipitate certain fractions of the plasma proteins, including fibrinogen, i.e., they may in themselves be directly thromboplastic. Thus samples of globin and of protamine have been encountered which cause an immediate coagulation of plasma by protein precipitation and "pseudoclot" formation. The mechanism of this coagulation in the case of globin has been previously outlined (11). The fact that certain preparations of protamine may evoke a similar precipitation phenomenon leads to the conclusion that the apparently heightened coagulability of the blood of normal humans (12) cannot be interpreted as due to neutralization of the "normal" heparin content extant.

The excess protamine content of certain protamine-insulin and protamine-zinc-insulin preparations[1] has been utilized by physicians for the control of capillary oozing even when the latter was probably not on the basis of hyperheparinemia. Such protamine-insulin preparations have a definite protein precipitant action. Modified human globin (Strumia, 13) which is devoid of such nonspecific protein precipitant action has been found not to affect the coagulation time of normal human subjects after intravenous administration.

SUMMARY AND CONCLUSIONS

Four pharmaceutic classes of antiheparinics have been tested for the *in vitro* restoration of spontaneous coagulability to heparinized human blood. The investigation was undertaken because of the prominent role which such therapy may assume in the hemorrhagic phases of atopic exudative syndromes such as the blood dyscrasias and radiation sickness.

In rapidity of action and *in vitro* effectiveness, toluidine blue is found to be superior to both hematoxylin and modified human globin.

Lactose, in solid phase, is antiheparinic but there appears to be no chance of the utilization of this property in practical antiheparin therapy.

REFERENCES

(1) Jacques, L. B., *Biochem. J.*, **37**, 189(1943).
(2) Jorpes, J. E., "Heparin: Its Chemistry, Physiology and Application in Medicine," Oxford University Press, 1939.
(3) Tocantins, L. M., *Proc. Soc. Exptl. Biol. Med.*, **54**, 94 (1943).
(4) Volkert, M., and Hertel, E., *Ugeskrift Laeger*, **105**, 784(1943).
(5) Barnard, R. D., and Sprinz, H., cited by Barnard, R. D., *Urol. and Cutaneous Rev.*, **51**, 46(1947).
(6) Barnard, R. D., Sprinz, H., Fitch, H. M., Lehman, R. A., and Walker, R. A., *Science*, **104**, 2706(1946).
(7) Barnard, R. D., *Ohio State Med. J.*, In Press.
(8) Allen, J. G., and Jacobson, L. O., *Science*, **105**, 388 (1947).
(9) Fisher, A., and Astrup, T., *Biochem. Z.*, **278**, 326 (1935).
(10) Holmgren, H., and Wilander, O., *Z. mikroskop.-anat. Forsch.*, **42**, 242(1937).
(11) Barnard, R. D., THIS JOURNAL, **34**, 177(1945).
(12) Fulton, J. F., "Howell's Textbook of Physiology," Philadelphia, 1946, p. 598.
(13) Strumia, M. M., Chornock, F. W., Blake, F. W., and Karr, W. G., *Am. J. Med. Sci.*, **209**, 436(1945).

WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

Ethyl acetamidomalonate
Thioindoxyl-2-carboxylic acid
Cholinesterase
Methylphenylbarbituric acid
2,2-Dichlorocamphane
3,4-Dihydroxynorephedrine
Creatinephosphate
Stevioside
N′-3,4-Dimethylbenzoylsulfanilamide
3-Carboxy-4-hydroxypyrazole
Corynanthine
Kephalin
d-Quercitol
Chondroitin sulfuric acid
β-(2-Pyrrole)acrylic acid
Quebrachitol
Indoxyl potassium sulfate
Dipeptidase
Nucleotidase
Porphyrexide
Hydroxytyramine

The Bactericidal Action of Oxygen-Liberating Substances on Oral Microorganisms. I. Studies with Fusiform Bacilli*

By THOMAS C. GRUBB and MARGUERITE A. EDWARDS

Two freshly isolated strains of fusiform bacilli, cultured in Brewer's thioglycollate medium, were inoculated into varying concentrations of sodium perborate, hydrogen peroxide, and a neutralized perborate dentifrice, diluted with water, saliva or saliva plus serum. Subcultures were made after thirty seconds, one, two, three, five, ten, and fifteen minutes' contact. The presence or absence of growth after seven days' incubation indicated the germicidal action of the substances tested. In a dilution of 1:5 the neutralized perborate dentifrice killed all the test organisms in one minute or less. After five minutes' contact the perborate dentifrice killed the test organisms in dilutions up to 1:40 in most instances. When sodium perborate and hydrogen peroxide were compared with the perborate dentifrice in various dilutions of equal oxygen content, hydrogen peroxide was more effective and sodium perborate less effective than the perborate dentifrice in the higher dilutions. Although all three products were equally effective in the lower dilutions usually employed for oral use, the strong alkalinity of sodium perborate and the high acidity of commercial hydrogen peroxide solutions are considered undesirable properties of products intended for oral application.

ALTHOUGH oxygen-liberating substances have been used for many years in the treatment of oral infections, especially Vincent's stomatitis, there appear to be few studies published on their bactericidal properties. Indeed, with regard to sodium perborate the United States Dispensatory (1) states, "It is rather extraordinary that despite the tremendous quantity of it which is used today, as far as we can find there is only one experimental investigation (2) concerning its bactericidal properties which has been published." Furthermore, so far as the writers are aware, no study has been published showing the *in vitro* action of oxygen-liberating compounds on the organisms associated with Vincent's infection—*Fusiformis dentium* and *Borrelia vincenti*. Possibly the reason for this situation is that fusiform bacilli and especially oral spirochetes are relatively difficult to isolate and culture so that they may be utilized for *in vitro* tests. Since the recent literature indicates methods for isolating and culturing fusiform bacilli (3) and oral spirochetes (4, 5) it now is practicable to determine the action of oxygenating agents on these organisms. The present report describes studies on the bactericidal action of hydrogen peroxide, sodium perborate, and a neutralized sodium perborate dentifrice[1] on fusiform bacilli.

* Received June 11, 1947, from the Research Laboratories, Vick Chemical Company, Flushing, N. Y.

EXPERIMENTAL

Two strains of fusiform bacilli (G. H. and F. N.) were isolated from normal, healthy mouths of two individuals by the method of Hine and Berry (3). These strains grew readily in Brewer's thioglycollate medium (6) and corresponded morphologically (see Fig. 1) and biochemically to *Fusiformis dentium* described by Hine and Berry. The number of fusiform bacilli in a given inoculum was readily determined by making a series of successively higher dilutions in Brewer's medium, incubating the tubes for twenty-four hours at 37° and counting the number of colonies in the tubes containing the higher dilutions. This is possible because the semisolid consistency of the medium permits the development of discrete colonies as shown in Fig. 2. Furthermore, the number of organisms surviving contact with an oxygenating agent can likewise be determined by counting the number of colonies developing in the subculture tubes.

In the first series of experiments an attempt was made to determine how rapidly fusiform bacilli were killed by the neutralized perborate dentifrice diluted with water, saliva, or saliva plus serum.

[1] The dentifrice (Neutrox) has the following composition: sodium perborate monohydrate (U. S. P. dehydrated), 30%; monocalcium phosphate, 30%; tricalcium phosphate, 23.87%; magnesium carbonate, 10%; saccharin soluble, 0.3%; sodium chloride, 2%; menthol, 0.33%; oil of peppermint, 0.5%; methyl salicylate, 0.65%; oil of anise, 0.042%; liquid petrolatum, light, 1.81%; and sodium lauryl sulfoacetate, 0.5%. U. S. patent No. 2,094,671.

TABLE I.—BACTERICIDAL ACTION OF THE NEUTRALIZED PERBORATE DENTIFRICE DILUTED 1:5 ON F. DENTIUM

Contact Time	Strain G. H. Water Dil.	Strain G. H. Saliva Dil.	Strain G. H. Saliva + Serum Dil.	Strain F. N. Water Dil	Strain F. N. Saliva Dil.	Strain F. N. Saliva + Serum Dil
30 sec.	21[a]	1	0	0	2	9
1 min.	0[b]	0	0	0	0	0
2 min.	0	0	0	0	0	0
3 min.	0	0	0	0	0	0
Control	22,960	320,000	320,000	2,700,000	8,000,000	8,000,000

[a] Figures indicate number of colonies in subculture tube. [b] 0 = no growth in subculture tube.

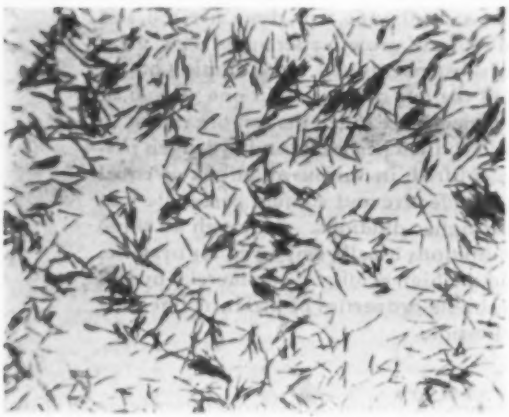


Fig. 1.—Fusiform bacilli from blood agar culture stained with gentian violet × 750.

In performing the test 0.5 cc. of a twenty-four-hour thioglycollate culture was added to 5 cc. of the dentifrice diluted 1:5 in distilled water, sterile saliva, or saliva plus 10% sterile rabbit serum. The tubes containing the diluted dentifrice and test organisms were kept in a 37° water bath and a 4-mm. loop was used to transfer a loopful of the mixture to tubes containing 20 cc. of thioglycollate medium after thirty seconds, one, two, and three minutes' contact. The subculture tubes were incubated at 37° for seven days before recording the absence of growth, number of colonies developing, or simply the presence of growth where the number of colonies was too numerous to count.

To determine whether absence of growth in the subculture tubes indicated a bactericidal or bacteriostatic action of the dentifrice, the following test was carried out a number of times. A loopful of a 1:5 dilution of the dentifrice was inoculated into a tube of thioglycollate medium followed by a loopful of the fusiform bacilli (diluted 1:11 to correspond with the concentration used in the actual test). When these tubes were incubated growth always developed promptly and profusely indicating that the "carry over" of the dentifrice into the subculture tube was insufficient to produce bacteriostatic conditions; or, that the sodium thioglycollate in the medium inactivated any sodium perborate that might be carried over by the loop. Hence it is believed that all of the tests reported in this paper where no growth was found in the subculture tubes may be construed as indicating that the fusiform bacilli were actually killed rather than merely inhibited from growing. An additional control test consisting of fusiform bacilli suspended in water up to five minutes at 37° showed that this treatment did not reduce the number of organisms.

In this first series of tests a 1:5 dilution of the dentifrice was chosen for testing because it has been estimated that this is the approximate concentration of the dentifrice in the mouth under the usual conditions of use.

The results shown in Table I indicate the extremely rapid bactericidal action of the neutralized perborate dentifrice. It should be noted that while the dentifrice killed some 320,000 G. H. organisms and 8,000,000 F. N. organisms, it should not be concluded that the dentifrice is less effective against the G. H. strain, for these figures simply indicate the number of organisms in the inoculum and quite possibly larger numbers of G. H. organisms would have been killed if they had been present in the inoculum. Inocula containing larger numbers of the G. H. strain were not tested. While the two fusiform strains appeared to be similar morphologically and biochemically, the F. N. strain usually grew much more luxuriantly than the G. H. strain so that higher concentrations of F. N. organisms were used as the inoculum in most tests. This may explain

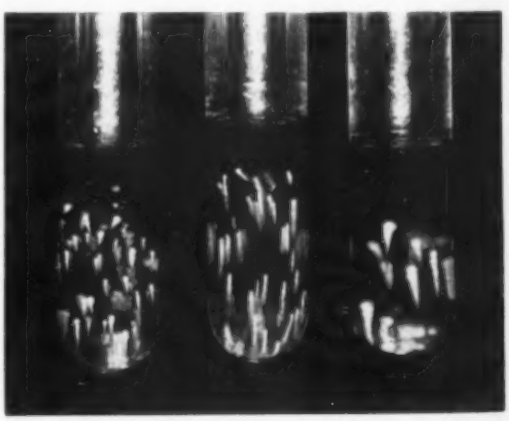


Fig. 2.—Fusiform bacilli growing in tubes of thioglycollate media. Note the discrete, stalactite-shaped colonies.

TABLE II.—BACTERICIDAL ACTION OF THE NEUTRALIZED PERBORATE DENTIFRICE DILUTED 1:10 TO 1:160 ON F. DENTIUM

Dilution	Water Dil. 5′	10′	15′	Saliva Dil. 5′	10′	15′	Saliva + Serum Dil. 5′	10′	15′
				Strain G. H.					
1:10	—[a]	—	—	—	—	—	—	—	—
1:20	—	—	—	—	—	—	—	—	—
1:40	—	—	—	28	—	—	+	—	—
1:80	85[b]	—	—	+	+	+	+	+	+
1:160	+[c]	+	20	+	+	+	+	+	+
				Strain F. N.					
1:10	—	—	—	—	—	—	5	—	—
1:20.	—	—	—	3	—	—	5	—	—
1:40	—	—	—	+	—	—	+	1	—
1:80	+	—	—	+	+	+	+	+	+
1:160	+	+	—	+	+	+	+	+	+

[a] — = no growth in subculture tube.
[b] Figures indicate number of colonies in subculture tube.
[c] + = Colonies too numerous to count.

why the F. N. strain often showed larger numbers of survivors than the G. H. strain in the subculture tubes, although the possibility that the F. N. organisms are more resistant *per se* than the G. H. organisms is not excluded.

The presence of saliva or saliva plus serum in the dilution mixture did not appear to have much effect on the bactericidal action of the dentifrice in this series of experiments.

Since the neutralized perborate dentifrice was so effective in low dilutions it was thought of interest to determine its action in higher dilutions and longer periods of contact with the test organisms.

Here again the neutralized perborate dentifrice shows a clear-cut bactericidal action in dilutions up to 1:40 in most instances. The difference between the results obtained with the two strains of fusiform bacilli is very marked in this series of tests. The F. N. strain was not killed so readily as the G. H. strain either because of the higher concentration of organisms in the inoculum or its inherent resistance, as noted above. The presence of saliva and serum caused some reduction in bactericidal activity.

Since sodium perborate or hydrogen peroxide is often used in dental practice to treat oral infections, it seemed of interest to compare their *in vitro* bactericidal action on fusiform bacilli with that of the neutralized perborate dentifrice. The test method was the same as that employed in the preceding experiments. In preparing the dilutions of the substances to be tested, the concentration of available oxygen per Gm. or cc. was first determined and then dilutions in water were made so that the corresponding dilution of each substance contained the same amount of available oxygen; e.g., a 1:50 dilution of sodium perborate and a 1:15 dilution of the neutralized perborate dentifrice contained equivalent amounts of available oxygen. The bactericidal tests were carried out within a few minutes after preparing the dilutions of each compound.

The results shown in Table III suggest that the neutralized perborate dentifrice is somewhat more

TABLE III.—COMPARISON OF BACTERICIDAL ACTION OF THE NEUTRALIZED PERBORATE DENTIFRICE AND SODIUM PERBORATE MONOHYDRATE ON F. DENTIUM

Perborate Dentifrice Dilutions	Perborate Dentifrice — Strain G. H. 5′	10′	15′	Strain F. N. 5′	10′	15′	Sodium Perborate Dilutions	Sodium Perborate — Strain G. H. 5′	10′	15′	Strain F. N. 5′	10′	15′
1:15	—[a]	—	—	—	—	—	1:50	—	—	—	—	—	—
1:30	—	—	—	—	—	—	1:100	—	—	—	—	—	—
1:60	—	—	—	—	—	—	1:200	11	—	—	4	—	—
1:120	7[b]	—	—	—	—	—	1:400	33	—	—	30	—	—
1:240	4	—	—	—	—	—	1:800	100	—	—	5	—	—

[a] — = no growth in subculture tube.
[b] Figures indicate number of colonies in subculture tube.

TABLE IV.—COMPARISON OF BACTERICIDAL ACTION OF THE NEUTRALIZED PERBORATE DENTIFRICE AND HYDROGEN PEROXIDE ON F. DENTIUM

Perborate Dentifrice Dilutions	Perborate Dentifrice — Strain G. H. 5′	10′	15′	Strain F. N. 5′	10′	15′	Hydrogen Peroxide Dilutions	Hydrogen Peroxide — Strain G. H. 5′	10′	15′	Strain F. N. 5′	10′	15′
1:10	—[a]	—	—	—	—	—	1:2.34	—	—	—	—	—	—
1:20	—	—	—	—	—	—	1:4.68	—	—	—	—	—	—
1:40	—	—	—	—	—	—	1:9.36	—	—	—	—	—	—
1:80	1[b]	—	—	—	—	—	1:18.72	—	—	—	—	—	—
1:160	6	—	—	4	3	—	1:37.44	—	—	—	—	—	—

[a] — = no growth in subculture tube.
[b] Figures indicate number of colonies in subculture tube.

effective than sodium perborate monohydrate when compared in the higher dilutions of equal available oxygen concentration.

When the neutralized perborate dentifrice is compared with hydrogen peroxide (U. S. P.), the latter appears more effective in the higher dilutions. It is possible that the greater activity of hydrogen peroxide may result from its low *p*H (3.7 to 4.9 in the dilutions tested), while the neutralized perborate dentifrice is essentially neutral, as demonstrated by Manchey and Lee (7).

In comparing the bactericidal action of hydrogen peroxide, sodium perborate, and the neutralized perborate dentifrice it is questionable whether the classical method of comparing germicides by determining the highest dilution which fails to show growth in the subculture tubes gives a fair indication of their relative value in actual usage. It is quite possible that all three substances are equally germicidal in the low dilutions in which they are usually employed in the mouth.

DISCUSSION

Although the results reported here are based on the use of but two strains of fusiform bacilli, it is believed that the bactericidal action of the oxygen liberating compounds was so consistent and clear cut that only slight variations in the results would be anticipated with a larger number of strains.

The dilution of the oxygenating agents with saliva and serum was, of course, intended to simulate *in vivo* conditions, and although there is some indication that these substances decrease the bactericidal activity in the higher dilutions tested, there is no significant reduction in activity in the dilutions ordinarily employed in oral application.

While it was pointed out above that in the dilutions ordinarily used in the mouth, hydrogen peroxide, sodium perborate, and the neutralized perborate dentifrice may have equal bactericidal action, other factors must be taken into consideration in evaluating the usefulness of any substance designed for oral application. Other factors being equal, it would appear desirable to employ a compound which was neither strongly alkaline, as is recognized in the case of sodium perborate (*p*H 9.9 in the dilutions tested), nor quite acidic as in the case of hydrogen peroxide, due to the oxygen preservative added to commercial preparations.

The wide usage of oxygen-liberating substances in the treatment of Vincent's infection appears to be based almost entirely on the satisfactory therapeutic results obtained in most cases. A recent study conducted in an Army hospital by Wihr (8) where cases of Vincent's infection were treated with penicillin by topical application or injection and the auxiliary use of an oxygen-liberating compound as a mouth wash, seems to indicate the continued use of oxygenating compounds in modern oral therapy. The *in vitro* demonstration of the rapid bactericidal action of oxygen-liberating substances on fusiform bacilli would appear to lend experimental support to the more or less empirical usage of these substances for the treatment of oral infections.

SUMMARY

Controlled bactericidal tests with sodium perborate, hydrogen peroxide, and a neutralized perborate dentifrice diluted with water, saliva, or saliva plus serum demonstrated the ability of these substances to kill oral fusiform bacilli rapidly and with approximately equal facility in the dilutions and simulated conditions of actual usage. The choice of these oxygen-liberating agents for oral application is discussed.

REFERENCES

(1) "U. S. Dispensatory," ed. 23, J. B. Lippincott Company, Philadelphia, Pa., 1943, p. 1003.
(2) Herhold, Von G., *Deut. med. Wochschr.*, **34**, 1092(1908).
(3) Hine, M. K., and Berry, G. P., *J. Bact.*, **34**, 517(1937).
(4) Wichelhausen, O. W., and Wichelhausen, K., *J. Dental Research*, **21**, 543(1942).
(5) Hampp, E. G., *J. Am. Dental Assoc.*, **30**, 1066(1943).
(6) Brewer, J. H., *J. Am. Med. Assoc.*, **115**, 598(1940).
(7) Manchey, L. L., and Lee, S., THIS JOURNAL, **26**, 890 (1937).
(8) Wihr, N. L., *J. Am. Dental Assoc.*, **34** 464(1947)

Chemical Investigation of *Premna Integrifolia* Linn.*

By N. K. BASU and P. C. DANDIYA

Two alkaloids, Premnine and Ganiarine, have been isolated from *Premna integrifolia* and their physical and chemical properties described.

Premna integrifolia Linn. or *Premna spinosa* Roxb. is a small tree or a big shrub belonging to the natural order Verbenaceae. It grows in India near the sea from Bombay to Malacca and Sylhet. It also grows in Ceylon. The common Indian names of the plant are Ganikarika (Sanskrit), Ganiari and Arni (Hindi, Bengali, and Gujrati), Munnay (Tamil).

The name "Ganiari" is very indiscriminately applied to another plant, *Clerodendron phlomoides* Roxb. belonging to the same natural order, but the latter is distinguished from the former by its vernacular synonym "Chhoti Arni" as the corresponding synonym for *P. integrifolia* is "Bari Arni."

The drug is very extensively used in the Ayurvedic system of medicine and the roots are considered to be laxative, stomachic and alexipharmic. The decoction of the roots is given in gonorrhea and during convalescence from fever. It is an important ingredient of "dasamula."

Regarding the chemical constituents of the drug, Dymock, Wardon, and Hooper (1) report the presence of an amorphous alkaloid, a substance reducing Fehling's solution, and an astringent body giving a green color with ferric chloride but giving no precipitate with gelatin. Beyond this, little work on the chemical constituents of the drug seems to have been done.

The present authors have been able to isolate two different alkaloids from the stem bark of the tree; one, Premnine, (empirical formula $C_{14}H_{15}NO$ and m. p. 82°) in pure amorphous form, from its pure crystalline hydrochloride; and the other, Ganiarine, only in the crude form. Ganiarine is extremely soluble in water, while premnine is only moderately soluble, which fact has formed the basis of separation of these alkaloids. Some derivatives of the bases have also been prepared. Some unsaturated hydrocarbons of high molecular weight have also been detected in the drug. The pharmacological action of premnine has been studied to some extent.

EXPERIMENTAL

Preliminary investigations with different solvents soon established the fact that the drug is best extracted with 90% alcohol by soxhlation. An acid solution of the crude alkaloidal bases is precipitated by Mayer's, Dragendorff's, Wagner's, and other alkaloidal reagents. Dragendorff's reagent is found to be the most suitable for the purpose.

Ten kilograms of the coarsely powdered drug was extracted with hot rectified spirit by soxhlation. The extract, on keeping, deposited some granular sediment. The clear supernatant liquid was decanted off and the sediment was reserved for investigation (A). The decantate was concentrated to a syrupy consistency and the hot syrupy liquid was slowly poured into 5 L. of warm hydrochloric acid (1%), with constant shaking. The mixture was shaken mechanically for two hours and filtered. A portion of the filtrate (500 cc.), representing 1 Kg. of the drug, was transferred to a separatory funnel and shaken with chloroform to remove the coloring matter, each chloroform extract being washed with the same 1% HCl kept in another separator. The acid liquid was then rendered alkaline with dilute ammonia and was shaken repeatedly with chloroform. It was observed that even 2 L. of chloroform could not extract the alkaloids completely. Extraction with chloroform was continued after successive addition of stronger alkalies like sodium carbonate and sodium hydroxide, but this also did not help in the complete extraction of the alkaloids. The aqueous liquid which still gave a strong test with Dragendorff's reagent was made neutral to litmus and reserved (B).

The rest of the acid extract was extracted with chloroform by the above process and the combined chloroform extract was dried over anhydrous sodium sulfate and filtered. The filtrate on distillation left behind a dark brown residue weighing 4.2 Gm.

Purification.—The residue was dissolved in a few cubic centimeters of chloroform and gasoline was added dropwise, whereby a lot of resinous matter precipitated. The supernatant liquid was decanted off and evaporated; the residue was again dissolved in a little chloroform and ether was added to it in excess, when black resins separated. The above process was repeated several times. The

* Received May 22, 1947, from the Department of Pharmaceutics, Benares Hindu University, Benares, India.

product finally obtained was yellow in color. Efforts were made to crystallize the base from various solvents, but it did not show a tendency to crystallize.

Preparation of the Hydrochloride.—The dry, yellowish semipurified alkaloidal residue was dissolved in dry ether and a few drops of absolute alcohol saturated with HCl gas were added to it drop by drop, when a yellowish precipitate was obtained. The precipitate obtained was dissolved in absolute alcohol and crystallized. The hydrochloride as finally obtained is in the form of colorless acicular crystals melting at 211–213°. The yield obtained was 65 mg. The name Premnine hydrochloride is suggested for it by the authors. A microphotograph of the hydrochloride is shown in Fig. 1.

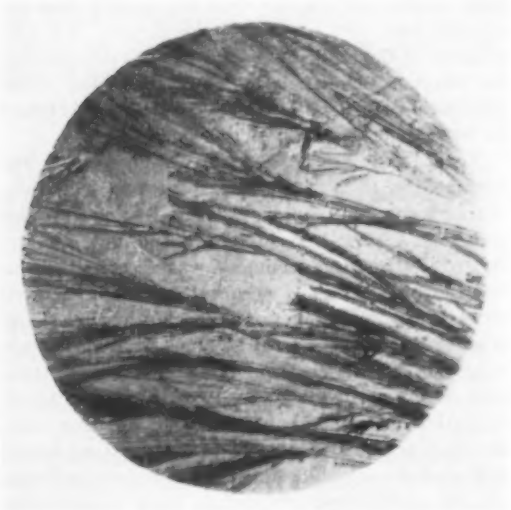


Figure 1

Preparation of the Base.—A portion of the hydrochloride was rubbed with moist silver oxide and a few cubic centimeters of water was added and the whole was again mixed thoroughly. The mixture was filtered and the filtrate on evaporation at 70° gave an amorphous base melting at 82°. It did not crystallize from any of the common solvents. The following derivatives were prepared: premnine picrate, m. p. 98–101° (with decomp.); premnine platinum chloride, decomposing at 254–256°.

On submitting the hydrochloride to semimicro combustion analysis, the following results were obtained: Carbon, 67.88; Hydrogen, 6.096; Nitrogen, 5.551; Chlorine, 14.41; Oxygen (by difference), 6.063.

By dividing the proportionate numbers of all the elements by the number of nitrogen, the empirical formula comes to $C_{14}H_{13}NO$. The empirical weight calculated from this formula is 213.

A weighed amount of the platinum chloride derivative was ignited in a microcrucible and the residue weighed. Assuming the base to be mono-acidic the molecular weight calculated from the data obtained is 212 and compares very closely with that obtained by calculation from the empirical formula.

Ganiarine.—To the neutral, aqueous solution "B" reserved previously was added an excess of strong solution of lead subacetate (B. P.) when a bulky precipitate of lead tannate was obtained. The precipitate was filtered off and H_2S gas was passed in the filtrate to precipitate the excess of lead as lead sulfide. After passing a current of air to remove the dissolved H_2S gas the bright yellow filtrate was evaporated to 50 cc. under reduced pressure. The concentrated extract was made alkaline with ammonia and again extracted with chloroform. It was observed that even 500 cc. of chloroform extracted very little alkaloid. The aqueous liquid was once again made neutral and evaporated to dryness *in vacuo*. The residue was extracted with absolute alcohol, which eliminated most of the salts formed during the previous processes. The alcoholic extract was concentrated and an excess of ether was added to it, when resins and more salts separated. The process was repeated several times and the product finally obtained by evaporation of the ethereal extract, was yellowish brown in color. Further purification of the alkaloid could not be attempted. The platinum chloride derivative which could be prepared from the crude base decomposes at 239–241°.

Hydrocarbons.—The granular sediment "A," reserved previously, on examination was found to be a mixture of unsaturated aromatic hydrocarbons of high molecular weight.

Pharmacology of Premnine

(a) **On Blood Vessels.**—The action of premnine on blood vessels was studied by determining the perfusion rate in a frog. First of all the number of drops of frog-Ringer Solution, flowing per minute was counted. Then 0.5 cc. of 1:5000 solution of premnine hydrochloride was injected into the rubber tubing connecting the reservoir of the frog-Ringer Solution to the ventricle of the frog and the number of drops per minute after the injection was counted. Three such readings were taken in each case. The mean of the readings was 37 and 27, respectively. The results show that the drug raises the blood pressure by contracting the blood vessels, i.e., a sympathomimetic drug.

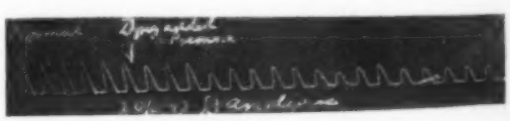


Figure 2

(b) **On the Heart.**—Heart of a pithed frog was exposed and frequently perfused with frog-Ringer

Solution. The ventricle of the heart was connected through a bent hook with the cardiograph lever attached with a small pointer touching the smoked paper fixed to the rotating drum. The curves indicating the normal contractions of the heart and also the contractions after perfusion with 1:5000 solution were recorded (Fig. 2). As is apparent from the recorded tracings the drug decreases the force of contraction of a frog's heart.

(c) **On the Eye.**—A drop of 1:10,000 solution of premnine on being put into one of the eyes of a frog showed dilation of the eye pupil. The same was observed in the case of a puppy.

SUMMARY

1. Two alkaloids—one, premnine in pure amorphous form (m. p. 82°, empirical formula $C_{14}H_{18}NO$) and the other, "ganiarine" only in crude form (platinum chloride derivative decomposes at 239–241°)—have been isolated from the stem bark of *Premna integrifolia*.

2. The pharmacological actions of premnine have been studied on blood vessels, heart, and eye of a frog.

3. Some unsaturated aromatic hydrocarbons of high molecular weight have also been detected in the drug.

REFERENCE

(1) Dymock, W., Warden, C. J. H., and Hooper, D. "Pharmacographia Indica," Part VI, London, 1893, p. 68.

The Estimation of Aloin in Pharmaceuticals*

By K. G. STONE†

A polarographic method for determination of aloin in pharmaceuticals has been applied to seven commercial preparations.

THE USE of aloin in the form of aloes or aloin isolated from aloes for pharmaceutical purposes is very old. There has been an obvious need for an analytical method for the estimation of aloin both in raw materials and in finished products. Previous methods have usually involved either extraction of the aloin or precipitation of calcium aloinate, both of which have been criticized on many points. Recently Stone and Furman (1) have utilized the polarographic method for the analysis of aloes. It is the purpose of this paper to describe the analysis of pharmaceuticals for aloin by this method.

THE POLAROGRAPHIC METHOD

The polarographic method may be briefly described as an electrolysis under very strictly controlled conditions so that the current flowing at any moment may be accurately measured. Organic groups such as carbonyl, nitro, azo, quinone, and conjugated double bonds are reducible, and the potentials required for the reduction of these groups are sufficiently different that the potential is characteristic for the particular group. The electrolytic measurements are made by measuring the current that flows at increasing potentials with a dropping mercury electrode and a quiet pool of mercury as the other electrode with no stirring of the solution. The mercury drop is usually the cathode with reduction occurring.

If the solution being electrolyzed contains an electrolyte, preferably a buffer solution to eliminate *p*H effects, no appreciable current flows until enough potential is applied to reduce something in the solution. As the potential increases, more current will flow until all of the material approaching the mercury drop is reduced. Then the current will become constant, since more material will approach the drop at a constant rate due to diffusion. The difference in current before and after this rise which is usually called "wave height" is proportional to the concentration of material being reduced.

A more detailed treatment may be found in Kolthoff and Lingane (2).

THE ESTIMATION OF ALOIN

Stone and Furman (1) found that there were no interfering substances in aloes, so that a direct determination could be made. (Obviously, any other substance that was reduced at the same potential as aloin would contribute to the increase in current, and thus interfere.) Commercial pharmaceuticals were analyzed by the same method with the results that follow.

* Received March 15, 1947, from the Frick Chemical Laboratory, Princeton University, Princeton, N. J.

† Present address: Kedzie Chemical Laboratory, Michigan State College, E. Lansing, Mich.

APPARATUS, MATERIALS, AND METHOD

A Leeds and Northrup Electrochemograph equipped with the cell arrangement described by Furman, Bricker, and Whitesell (3) was used for the polarographic measurements at room temperature. The capillary had the following characteristics: m = 0.8173 mg. per second, t = 7.60 seconds at −1.34 volt against the saturated calomel electrode (S. C. E.) in the buffer solution; the head of mercury was 46.5 cm. Oxygen was removed by passing purified nitrogen through the solution for fifteen minutes before polarographing. All polarograms were taken at 1/50 sensitivity.

The 0.25 *M* acetate buffer of *p*H 4 was prepared by neutralizing 0.25 mole of glacial acetic acid in 600 ml. of water with concentrated sodium hydroxide solution to *p*H 4 measured against a glass electrode and diluting to 1000 ml. A polarogram of the buffer alone showed no appreciable amount of reducible material before hydrogen evolution.

Merck U. S. P. aloin was used without further purification. For a standard solution, 1.000 Gm. of aloin was dissolved in 1000 ml. of 20 volume per cent ethanol. This solution was stable for about one week when protected from light and air.

Procedure.—Weigh about 1.5-Gm. sample to the nearest milligram (for aloes, 0.2 Gm. to the nearest 0.1 mg.). Warm in 20 ml. 95% ethanol and 25 ml. water for fifteen minutes just below the boiling point. Macerate with stirring rod if necessary. Cool to room temperature, filter on paper into a 100-ml. volumetric flask, wash the residue with three 10-ml. portions of distilled water collecting the wash solution with the original filtrate, and dilute to the mark. Mix a 10-ml. aliquot with 10 ml. of the acetate buffer and dilute to 25 ml. Deaerate with nitrogen, and take a polarogram from −1.0 volt applied potential. Compare the wave-height with the calibration curve.

Preparation of the calibration curve: Mix suitable volumes of the standard aloin solution with 10 ml. of the acetate buffer, add ethanol to give a total concentration of eight volume per cent ethanol in the final solution, and dilute to 25 ml. Proceed as with the sample. The calibration curve is usually a straight line for concentrations up to 15 mg. of aloin per 25 ml. of the solution polarographed. The most critical factor is the ethanol concentration, since the reducible species is quite dependent on the solvent composition. If the composition is not constant, the calibration curve will not be a straight line. It was also noticed that an old standard solution of aloin also led to deviations from a straight line calibration. Various lots of aloin seemed to have very little effect on the current-concentration ratio.

In order to check the working procedure, a series of recovery studies was made. A sample of pure barbaloin was prepared according to the procedure of Gardner and Joseph (4). This barbaloin was used as a reference standard for all recovery studies of which the following are typical:

1. A sample of aloin equal to 10.0 mg. of the pure barbaloin was analyzed and 10.2 mg. of barbaloin were found.
2. A mixture of 5.0 mg. of barbaloin and aloes equivalent to 4.2 mg. of barbaloin was found to contain 9.1 mg. of barbaloin.
3. A pharmaceutical which was said (5) to contain 5 mg. of aloin per tablet was analyzed and 4.7 mg. per tablet were found.

RESULTS

In Table I will be found the per cent of aloin found in each of seven preparations. These products are from seven different manufacturers, and consist of various mixtures of aloes or aloin, soap, assorted gums, podophyllin, extracts of belladonna, stramonium, etc., ipecac, phenolphthalein, and flavors. It is not possible to tell the amount of aloes present in the preparations containing aloes unless the relation between aloin and aloes is known since the amount of aloin in aloes varies from lot to lot depending on the source. Hence, in order to compare the preparations, all of which were recommended for treatment of colds, the assay was combined with the recommended dose on the label to give the recommended dose in terms of aloin. It is interesting to note that the dose as aloin varies 25% from the average.

TABLE I.—DETERMINATION OF ALOIN IN PHARMACEUTICALS

Preparation	% Aloin	Dose, Gm. Aloin
A	6.1	0.0138
B	7.5	0.0224
C	7.9	0.0225
D	9.5	0.0355
E	13.8	0.0335
F	18.1	0.0172
G	24.6	0.0207
		Av. 0.0237

Very few materials are known that are reduced in this potential range that also occur in the pharmaceutical raw materials used. Hence an interference in this determination is possible, but not probable. The usual polarographic analysis has an inherent error of about three relative per cent so that error may be anticipated in this determination.

REFERENCES

(1) Stone, K. G., and Furman, N. H., *Anal. Chem.*, **19**, 107(1947).
(2) Kolthoff, I. M., and Lingane, J. J., "Polarography," Interscience Publishing Co., New York, 1941.
(3) Furman, N. H., Bricker, C. E., and Whitesell, E. B., *Ind. Eng. Chem., Anal. Ed.*, **14**, 33(1942).
(4) Gardner, J. H., and Joseph, L., THIS JOURNAL, **26**, 794(1937).
(5) Private communication from the manufacturer.

Pharmacognostical Studies of Brahmi

Stem and Leaf Characteristics of *Herpestis monniera* H. B. and K. and *Hydrocotyle asiatica* Linn.*

By S. PRASAD†

This report includes new information on the pharmacognosy of the drug known in India as Brahmi. The results reported will serve to distinguish between the two plant species involved in connection with Brahmi.

A VERY important drug in the Ayurvedic Materia Medica of India, Brahmi has been used by the Hindu physician as a nervine tonic from very ancient times. It has been considered useful also in epilepsy and insanity and has been presented to the Western physician, practicing in India, in the form of syrup, liquid extract, ghee, and hair oil by many pharmaceutical manufacturing houses of that country.

The correct identity of this reputed drug has, however, not yet been ascertained. While one group of Ayurvedic physicians regards *Herpestis monniera* H. B. and K. (Fam. Scrophulariaceae) to be Brahmi, there are others who consider *Hydrocotyle asiatica* Linn. (Fam Umbelliferae) as the real drug. Recently Sircar (1) tried to establish, on the basis of botanical characters embodied in the Ayurved that *Hydrocotyle asiatica* and not *Herpestis monniera* is the real Brahmi. In the drug market both these species are sold indiscriminately as Brahmi.

A fair amount of chemical and pharmacological work has already been done on these two plant species (2–4). No pharmacognostical study on any of these plant species has yet been brought on record. The present investigation was, therefore, taken up with a view to bring out all those diagnostic features which may help one to distinguish one species from the other. The anatomy of the leaf and of the stem, along with their macroscopical characteristics, has been studied in detail in both the species. The investigation has been extended to the determination of their stomatal indices and palisade ratios as well, to ascertain if these values could be utilized for distinguishing the two species.

* Received June 24, 1947.

† Of the Department of Pharmaceutics, Benares Hindu University, Benares, India; now at the School of Pharmacy, University of Wisconsin, Madison, Wis.

MATERIALS AND METHODS

Fresh specimens of *Herpestis monniera* and *Hydrocotyle asiatica* were collected from the Ayurvedic garden of the Benares Hindu University. Free hand sections from the different portions of stem, leaf, and petiole were stained in safranin and gentian violet and mounted in Canada balsam in the usual way. Surface peelings were examined in chloral hydrate solution. The palisade ratio and stomatal index were determined by the following procedure:

Pieces of leaf tissue, 1–3 mm. square, were cut from the base, apex, and the middle portions of the leaf and placed in test tubes containing concentrated chloral hydrate solution (50 Gm. chloral hydrate dissolved in 20 cc. water). These were then placed in a water bath for about twenty minutes or more until they became more or less transparent. The palisade ratio was then determined according to the method employed by Wallis and Dewar (5).

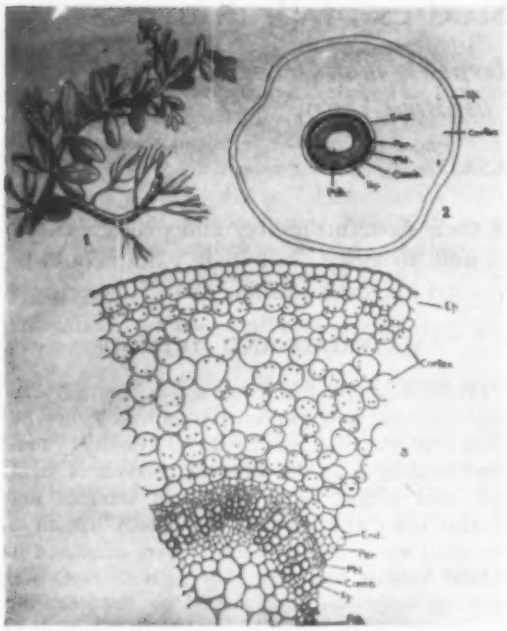
For determining stomatal index, similar procedure was adopted so far as the clearing of leaves was concerned. The stomatal indices were found as proposed by Salisbury (6), that is, $I = S/(E + S) \times 100$, where S is the number of stomata per unit area, and E the number of epidermal cells in the same unit area. During the process of determination of stomatal values, two guard cells were considered as one unit, each hair one unit, and each epidermal cell one unit. To determine if the stomatal index and palisade ratio varied significantly from locality to locality, the data pertaining to these were statistically analyzed to test the significance of the result.

INVESTIGATION

External Morphology

Herpestis monniera.—It is a creeping, somewhat succulent herb, found in moist places near streams or on the banks of ponds and ditches. It grows throughout the plains of India, ascending to 4000-ft. altitude on the Himalayas and in all warm countries.

Several stems, 4–12 inches long, arise from the main stem. The plant roots at the nodes. The branches are ascending. The leaves are sessile, decussate, 1/6–1 inch long, 0.20–0.35 inch broad,



Herpestis monniera H. B. K.

Fig. 1.—The whole plant with flowers. × 1/3.
Fig. 2.—Plan of the transverse section of the stem. × 20.
Fig. 3.—Details of transverse section. × 65.
Ep., epidermis; End., endodermis; Per., pericycle; Phl., phloem; Camb., cambium; Xy., xylem.

obovate-oblong or spatulate, entire, nerves obscure and lower surface dotted (Fig. 1). Flowers are axillary and solitary, borne on slender pedicels 1/6–1 1/4 inches long. Bracteoles are linear, shorter than the pedicels. Calyx is divided to the base, glabrous, upper segments ovate acute, longer and broader than the other. Corola is 1/3 inch long, blue or white with purple veins; lobes are nearly equal and rounded, glistening with shining dots when fresh. Capsules are 1/5 inch long, ovoid, acute, and glabrous.

Hydrocotyle asiatica.—It is an annual herb, creeping along the ground and rooting at the nodes (Fig. 11). It grows throughout India, Ceylon, and Malaya and in all the tropical and subtropical regions of the world.

Leaves, in the Indian species, are cordate or hastate, orbicular or reniform, crenate and often palmately lobed. They are 1/2–2 1/2 inches each way, glabrous or nearly so and shining, with stipules adnate to the petioles. Peduncles are much shorter than the leaves, often 2–3-nate, pubescent. Bracts are small, ovate, embracing the flowers and not scattered among the pedicels (Fig. 11 (*A*)). Flowers are sessile and grouped 3–6 in each head. Petals are minute, ovate, acute, and slightly imbricate; carpels oblong, subcylindrical, curved, much longer than broad, slightly compressed, reticulate-rugose, each with 9 curvilinear, subsimilar ridges and 2 within the commissure. The fruit is 1/6–1/4 inch in diameter (Fig. 11 (*B*)). Pericarp is thickened, woody, and white (Fig. 11 (*C*)).

Microscopical Features

Herpestis monniera

Stem.—The transverse section of the stem indicates that it consists of a single layer of epidermis, followed by a wide parenchymatous cortex and a ring of stele which encloses in the center a small amount of pith (Fig. 2). The epidermis is covered externally by a thin layer of cuticle. The epidermal cells are small and cubical and measure 20–30–38 microns × 24–34–40 microns.[1]

Beneath the epidermis is a wide zone of cortex which is composed of thin-walled, isodiametric and almost round cells with very large intercellular spaces that serve as air chambers (Fig. 3). The cortex is thus lacunar. The cortical cells measure 30–51–85 microns in diameter. The bigger cells occupy the middle region of the cortex and are in some cases tangentially elongated. The innermost layer of the cortex is represented by a distinct endodermis. The endodermal cells show Casparian strips in cross section and measure 28–35–45 microns × 18–30–35 microns.

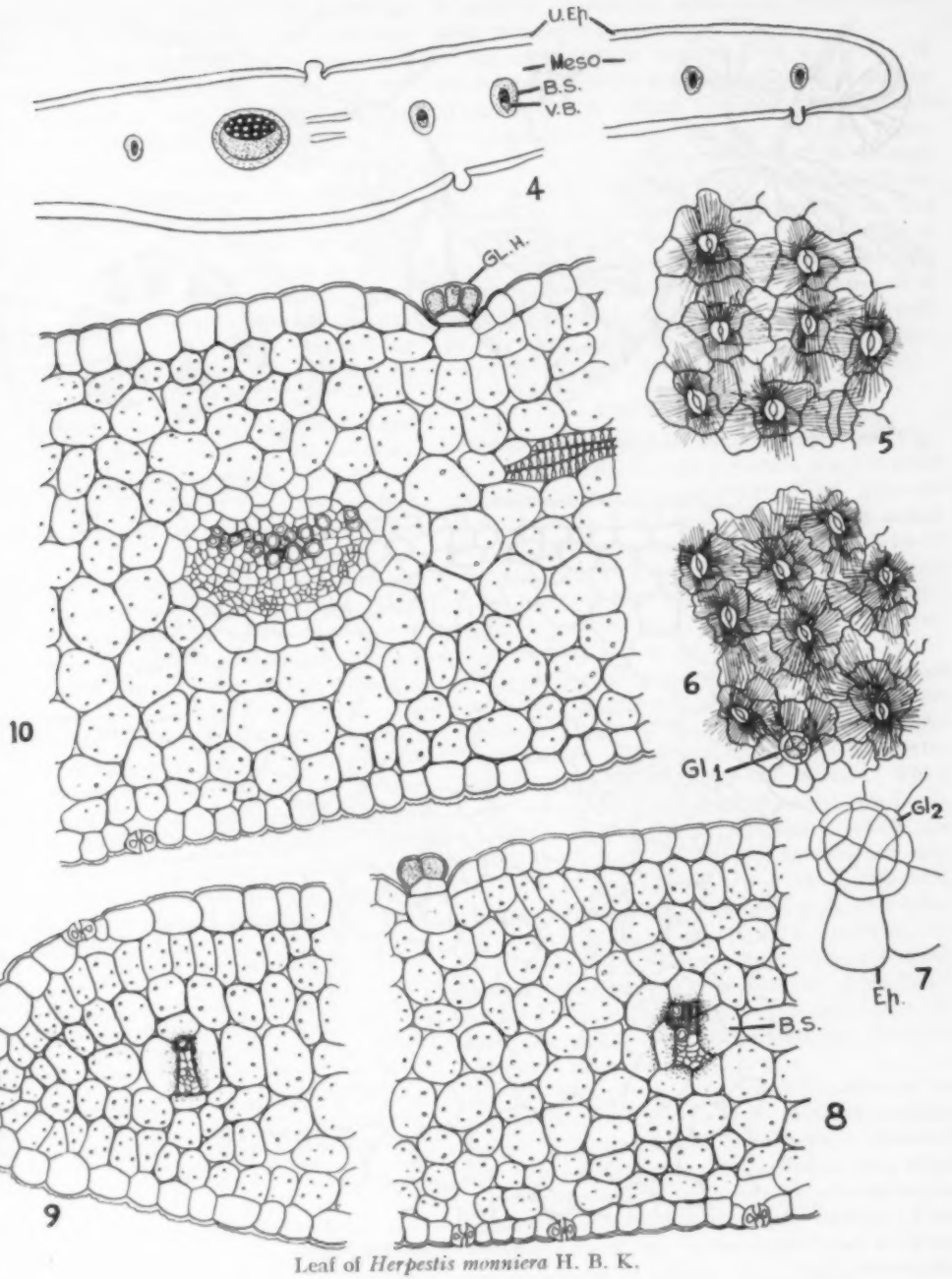
Following the endodermis is a thin strip of pericycle, consisting of 1–2 layers of cells (Fig. 3). The pericycle surrounds the stele all around and the pericyclic cells are very small, measuring 12–15–20 microns in length and 9–12–17 microns in breadth.

The vascular ring is continuous, being composed of a narrow zone of phloem toward the peripheral side, a wide ring of xylem toward the center, and a layer of cambium separating the two. The phloem consists of sieve tube tissue associated with companion cells and phloem parenchyma. The xylem is composed of xylem vessels with intervening xylem parenchyma. The vessels are polygonal and isodiametric and arranged in radial rows. They are 14–22–30 microns in diameter in transverse section. The xylem parenchyma consists of thin-walled cells, measuring 8–13–17 microns in length and 7–11–14 microns in breadth.

In the center is a small pith, the cells of which are thin walled, rounded, more or less isodiametric with distinct intercellular spaces. They are 28–38–55 microns in diameter.

Leaf.—The structure of the leaf of *Herpestis monniera* resembles more an isobilateral type of leaf than a dorsiventral one, in that it shows a lack of differentiation of the mesophyll in the palisade and spongy layers (Figs. 8–10). The surface of the leaf is covered externally with cuticle which occurs in the form of striations. These striations are more strongly developed on the lower surface than on the upper one. In surface view, the arrangement of the cuticular striations is such that they appear to

[1] The measurements of cells and other structures are given ordinarily in three separate figures, of which the first and the third represent the minimum and the maximum values, respectively, while the middle indicates the common value.



Leaf of *Herpestis monniera* H. B. K.

Fig. 4.—Transverse section of the leaf. × 45.

Fig. 5.—Upper epidermis in surface view. × 180.

Fig. 6.—Lower epidermis in surface view. × 180.

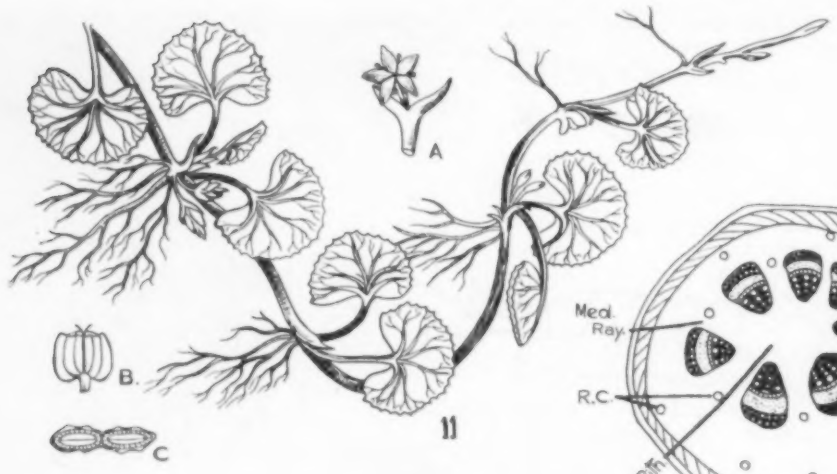
Fig. 7.—Glandular hair in surface view. × 450.

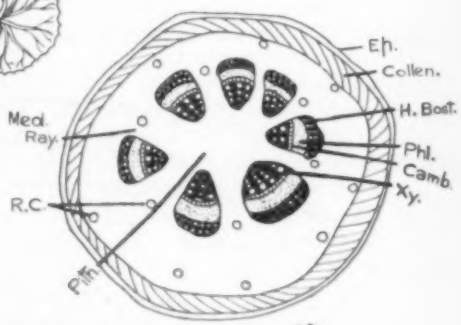
Fig. 8.—Details of transverse section of the leaf. × 225.

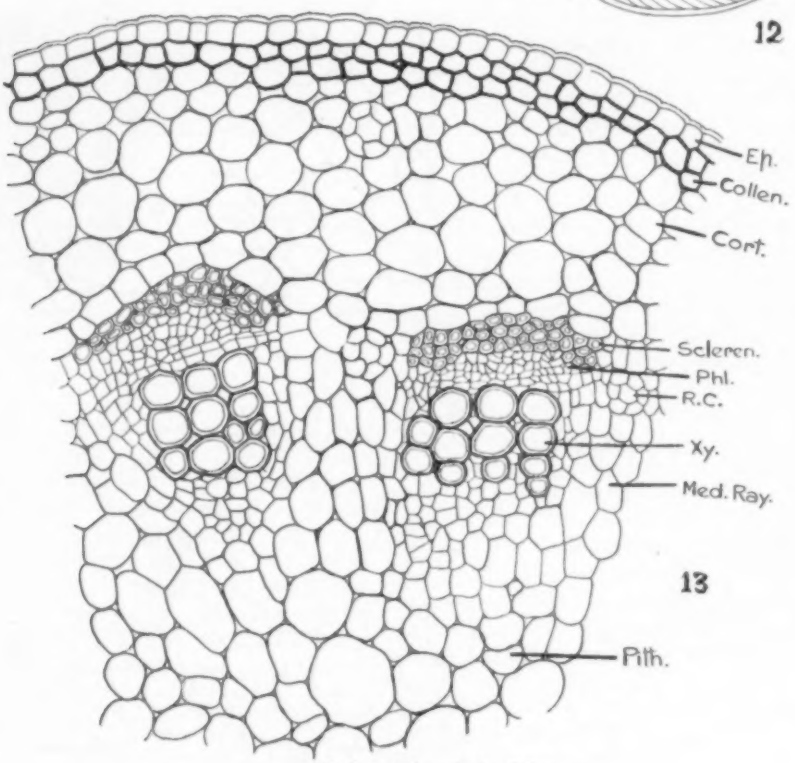
Fig. 9.—Transverse section of the margin of the leaf. × 225.

Fig. 10.—Transverse section passing through the midrib. × 225.

U.Ep., upper epidermis; Meso, mesophyll; B.S., bundle sheath; V.B., vascular bundle; Gl.H., glandular hair; Gl_1, glandular hair in surface view, showing quadrucellular head; Gl_2, the same with a head of 8 cells in surface view.







Hydrocotyle asiatica Linn.

Fig. 11.—A part of the plant. × 3/4. *A*, inflorescence. × 2 1/2. *B*, fruit. × 3. *C*, fruit cut across. × 3.

Fig. 12.—Transverse section of the stem. × 40.

Fig. 13.—Details of transverse section. × 225.

Ep., epidermis; Collen., collenchyma; Cort., cortex; Scleren., sclerenchyma; Phl., phloem; R.C., resin canal; Xy., xylem; Med. Ray, medullary ray; H. Bast, hard bast; Camb., cambium.

be diverging away from stomata in all directions (Figs. 5 and 6). In transverse section, the striations are to be seen as small ridges which are more pronounced toward the margins of the leaves (Fig. 9).

Stomata are found on both the surfaces of the leaf. There are no subsidiary cells surrounding the stomata. According to Vesque (7) their development follows the cruciferous type, but this is frequently obscured in the mature leaf; that is to say, the stomata are surrounded by three or more epidermal cells. In surface view the stomata of the upper surface (Fig. 5) measure 25–28–30 microns in length and 18–20–23 microns in breadth with the aperture being 4–6 microns wide. The stomata on the lower surface (Fig. 6) are more or less of similar dimensions as noted in the case of the upper ones.

Epidermal cells, in surface view, are much more wavy walled on the lower surface than on the upper one (Figs. 5 and 6). They measure 34–51–64 microns × 20–30–38 microns on the upper surface and 38–50–68 × 22–28–34 microns on the lower side.

Glandular hairs are present on both the surfaces. They occur in slight depressions of the lamina (Figs. 8 and 10). Some of them are smaller than others. The smaller ones are round or oval and quadricellular, borne upon a slightly conical stalk, while the larger ones possess the glandular head of eight cells. The epidermal cell joining the stalk bulges out and helps in the elongation of the stalk. In surface view the hairs are seen as circles divided into four or eight cells (Figs. 6 and 7). The glandular heads have a diameter of 28–35–44 microns. The distribution of these hairs, stomata, and epidermal cells per sq. mm. leaf surface (Table I) indicates that the upper surface of the leaf has comparatively more hairs and less stomata than the lower one.

TABLE I.—NUMBER OF STOMATA, HAIRS, AND EPIDERMAL CELLS PER SQ. MM. LEAF SURFACE IN *Herpestis monniera*

Surface	Stomata	Hairs	Epidermal Cells
Upper	65–95–128	20–32	473–615–717
Lower	90–115–141	13–26	485–620–757

In transverse section the epidermal cells are somewhat vertically elongated, measuring 14–24–35 microns × 30–35–42 microns in case of the upper surface and 15–30–35 microns × 20–25–30 microns on the lower surface. The palisade tissue is weakly developed, the mesophyll cells being 35–45–60 microns in length and 22–30–40 microns in breadth. The spongy mesophyll, in the center, is rather more compactly arranged and the cells are bigger and more or less isodiametric with a diameter of 30–56–80 microns. Toward the dorsal side, the spongy layer is somewhat loosely arranged and the cells are smaller in dimensions. This meager difference between the palisade and spongy cells is still less marked in the margin region (Fig. 9). A few prismatic crystals of calcium oxalate are occasionally found distributed in the mesophyll cells.

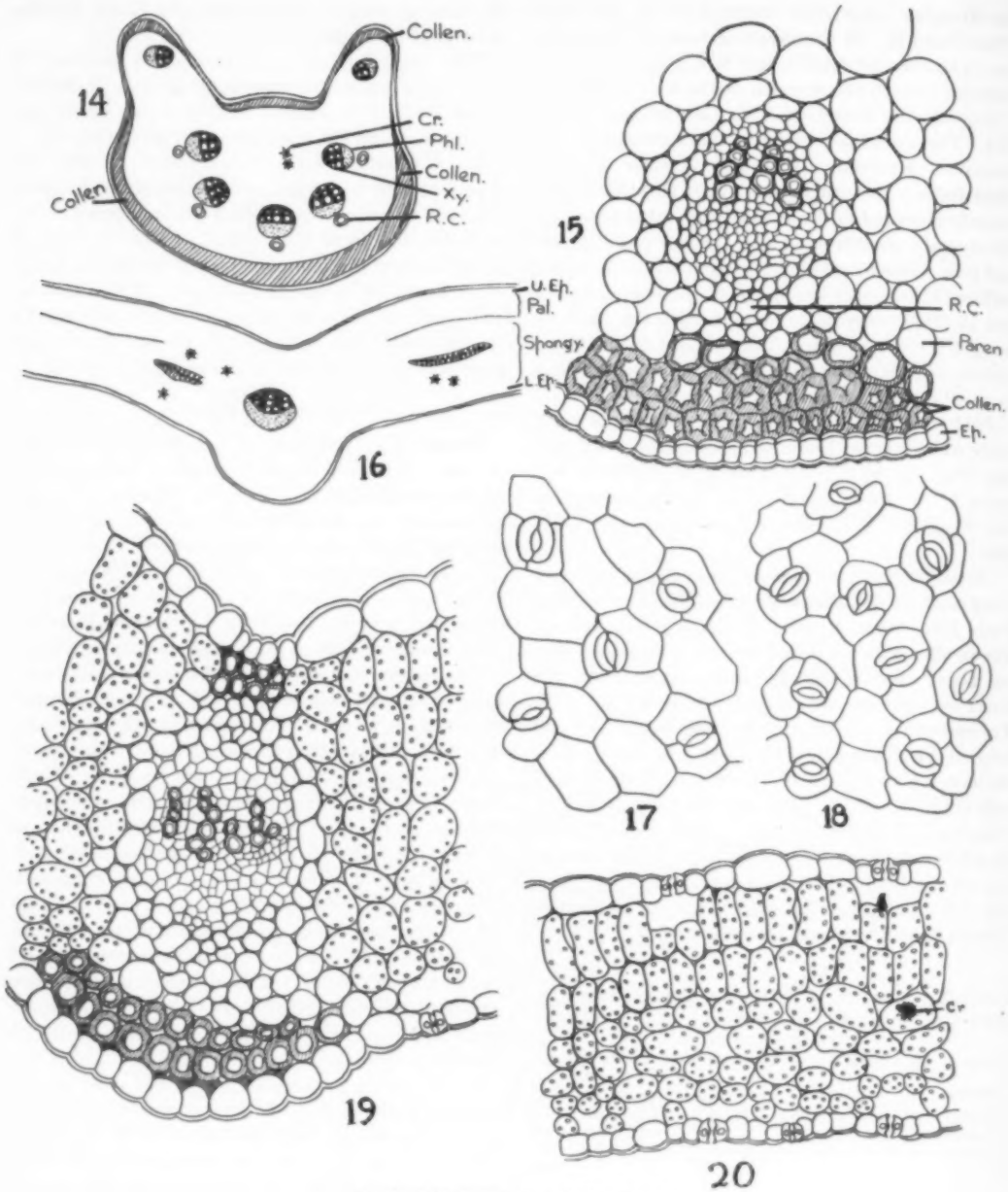
The mesophyll is traversed by a number of small veins which are surrounded by bundle sheaths (Figs. 8–10). A distinct midrib, is, however, not to be found and the transverse section in this region (Fig. 10) indicates the arrangement of different layers of cells to be the same as mentioned above. There is no collenchyma below the epidermis. The vascular bundle of the midrib as also of those of other veins of the lamina is poorly developed, being represented by a few annual and spiral vessels in the xylem on the ventral side and a few phloem cells on the dorsal side. A distinct bundle sheath surrounds the vascular bundle of the midrib as well.

Hydrocotyle asiatica

Stem.—The transverse section of the stem (Figs. 12 and 13) shows on the outside a single-layered epidermis covered externally by cuticle. The epidermal cells are small, cubical, and convexly arched outward, measuring 14–18–22 microns × 10–14–17 microns. Following the epidermis is a collenchyma of 2–3 layers of cells which are more or less isodiametric, measuring 14–17–22 microns in diameter. The remaining part of the cortex is occupied by 6–7 layers of parenchymatous cells which are much larger than the collenchymatous cells and measure 25–38–50 microns in diameter. A distinct endodermis is, however, not easily made out. The cortex encloses a ring of seven vascular bundles. These have toward the periphery strongly developed, semilunar patches of sclerenchyma which are stained red by phloroglucin and hydrochloric acid. The vascular bundle is of the common, collateral, and open type. The phloem consists of sieve tubes with companion cells and phloem parenchyma and the xylem consists of wood vessels, tracheides, and xylem parenchyma. The protoxylem elements are arranged toward the inner side, the metaxylem toward the periphery. The vessels are 17–30–35 microns in diameter.

In the center is the pith, which has more or less rounded isodiametric cells with distinct intercellular spaces, measuring 34–50–70 microns in diameter. The pith joins the cortex by medullary rays which are characterized by the possession of resin canals, usually one in between two vascular bundles. Resin canals are found also in the cortical region. These are surrounded by a layer of 6–7 small, thin-walled epithelial cells and the central duct is 25–35 microns in diameter.

Petiole.—The petiole in transverse section (Figs. 14 and 15) has a characteristic outline, due to the sides projecting out from the ventral groove. The epidermis is covered externally by a cuticle and has its inner walls adjoining the cortex, greatly thickened. The cells are somewhat cubical and measure 15–17–20 microns × 10–15–18 microns. Beneath the epidermis is a collenchymatous ring of 2–3 layers of cells. The collenchyma is, however, absent on the sides of the projecting arms of the petiole



Leaf of *Hydrocotyle asiatica* Linn.

Fig. 14.—Transverse section of the petiole. × 40.

Fig. 15.—Details of the same. × 225.

Fig. 16.—Transverse section of the leaf. × 40.

Fig. 17.—Upper surface showing the distribution of stomata. × 225.

Fig. 18.—Lower surface showing the distribution of stomata. × 225.

Fig. 19.—Transverse section passing through midrib. × 225.

Fig. 20.—Details of the transverse section of the leaf. × 225.

Ep., epidermis; Collen., collenchyma; Paren., parenchyma; R.C., resin canal; U.Ep., upper epidermis; Pal., palisade layer; Spongy, spongy layer; L.Ep., lower epidermis.

(Fig. 14). These cells have great angular thickenings (Fig. 15) and are polygonal, measuring 17–25–34 microns × 17–22–30 microns. Following the collenchyma is a broad zone of parenchyma, the cells of which are more or less round, showing prominent intercellular spaces and measure 25–50–68 microns in diameter. Within this zone are to be found seven vascular bundles, two of which are present in the projecting arms of the petiole, while the remaining five form a dorsal arc. Usually there is a resin canal situated on the dorsal side of these vascular bundles. Some of the parenchymatous cells contain rosette crystals of calcium oxalate, measuring 8–15 microns in diameter.

Leaf.—The leaf has a structure of the typical dorsiventral leaf, with its mesophyll differentiated into an upper palisade and a lower spongy tissue (Figs. 16 and 20). The surface of the leaf is covered with a thin, striated cuticle which is more strongly developed on the midrib. Stomata occur on both the surfaces (Figs. 17 and 18). They are usually surrounded by three subsidiary cells, but occasionally four or five cells also accompany them. Of the three subsidiary cells, two are often placed opposite the stomatal aperture, more or less parallel, while the third is at right angles to the pore. The stomata are 28–31–34 microns long and 24–26–28 microns broad with the stomatal aperture being 6–10 microns wide. Hair is absent in this leaf. The distribution of stomata and the number of epidermal cells per sq. mm. leaf surface (Table II) indicate that the lower surface has greater numbers of these than the upper one.

TABLE II.—NUMBER OF STOMATA AND EPIDERMAL CELLS PER SQ. MM. LEAF SURFACE OF *Hydrocotyle asiatica*

Surface	Stomata	Epidermal Cells
Upper	104–140–218	744–805–845
Lower	180–220–243	825–956–1027

In surface view the epidermal cells are all polygonal with straight walls. They are bigger on the upper surface than on the lower one (Figs. 17 and 18) and measure 24–58–85 microns × 20–32–46 microns on the ventral side and 18–52–75 microns × 17–28–40 microns on the dorsal side.

In transverse section (Fig. 20) the epidermis has somewhat tangentially elongated cells which are larger on the upper side than on the lower one. They are 15–22–32 microns × 12–15–20 microns on the upper surface and 9–14–20 microns × 6–10–15 microns on the lower surface. Beneath the upper epidermis are 2–3 layers of palisade tissue. The upper palisade layer consists of larger cells, measuring 38–50 microns × 17–25 microns. The spongy mesophyll is represented by 5–7 layers of loosely arranged and somewhat isodiametric cells which are bigger in the upper layers with a diameter of 17–35 microns, while the lower layers possess a diameter of 12–18–28 microns. A few cells of the spongy layer also contain rosette crystals of calcium oxalate, measuring 8–15 microns in diameter.

The midrib has a characteristic structure in the transverse section (Fig. 19). The epidermal cells, particularly on the dorsal side, are cubical. Below the epidermis is found a collencyma of 2–3 layers of cells on both the sides, following which are 4–5 layers of parenchymatous cells, mostly devoid of chloroplasts. The central region is occupied by a well-developed vascular bundle with phloem toward the dorsal side and the xylem on the ventral side. As usual the phloem consists of sieve tube tissue with companion cells distributed in the phloem parenchyma, while the xylem is composed of radial rows of xylem vessels with intervening xylem parenchyma. No distinct bundle sheath surrounds the vascular bundle either of the midrib or of the lamina.

To elucidate further the diagnostic differences of these two species, their stomatal indices and palisade ratios have been studied (Tables III and IV).

TABLE III.—STOMATAL INDICES OF *Herpestis monniera* AND *Hydrocotyle asiatica*

	Herpestis monniera		*Hydrocotyle asiatica*	
	Upper Surface	Lower Surface	Upper Surface	Lower Surface
	12.77	17.25	20.51	17.32
	12.50	16.44	14.56	16.86
	13.76	17.35	11.58	17.26
	13.98	16.64	15.46	17.65
	15.96	15.78	14.45	16.57
	12.46	17.65	14.84	17.56
	14.11	17.20	14.83	17.56
	12.95	16.27	15.24	21.28
	11.50	16.02	14.32	17.64
	13.17	14.95	14.43	16.71
	12.76	14.75	16.12	15.92
	15.11	16.58	15.43	19.43
	14.34	17.45	14.56	18.25
	13.70	15.58	14.25	19.43
	13.38	17.65	13.46	19.43
	12.24	16.44	14.53	17.64
	13.23	17.25	15.27	21.05
	15.10	15.80	14.56	17.58
	12.05	14.95	15.25	19.56
	14.35	16.55	14.58	16.68
Mean	13.50	16.42	14.93	17.89

Analysis of Variance

Variation Due to	Degrees of Freedom	Sum of Squares	Mean Sum of Squares
Species	1	43.3777	43.3777
Surface	1	179.3065	179.3065
Surface × species	1	0.11025	0.11025
Error	76	152.34355	2.00452
Total	79	375.13800	

The analysis of variance shows that highly significant variation exists between these two species. Variation due to surface is also significant. This is because great differences exist in the distribution of stomata on the upper and the lower surfaces, per sq. mm. leaf area, particularly in the case of *Hydrocotyle asiatica*, where the lower surface happens to

possess at an average 50–60% more stomata than the upper one. The interaction between surface and species is not significant. These two species therefore can be distinguished easily by their stomatal indices, since *Hydrocotyle asiatica* possesses a much higher value than *Herpestis monniera* (Table V).

TABLE IV.—PALISADE RATIOS OF *Herpestis monniera* AND *Hydrocotyle asiatica*

Herpestis monniera		*Hydrocotyle asiatica*	
1.25	1.50	3.75	3.50
1.75	1.25	5.00	5.25
2.25	2.25	5.75	4.00
2.00	2.00	3.25	3.50
2.25	2.00	4.00	5.75
2.00	2.25	5.25	3.75
1.75	2.00	4.00	4.00
2.25	1.75	4.75	5.25
1.50	1.25	5.00	4.50
2.00	1.75	4.00	5.50
Mean	1.85		4.46

Analysis of Variance

Variation Due to	Degrees of Freedom	Sum of Squares	Mean Sum of Squares
Species	1	68.2516	68.2516
Error	38	19.0834	0.5022
Total	39	87.335	

It will be seen from the above table of variance that very high significant variation exists between the two species. Thus they can be differentiated on the basis of their palisade ratios also.

TABLE V.—RANGE OF VARIATION IN THE STOMATAL INDICES AND PALISADE RATIOS OF *Herpestis monniera* AND *Hydrocotyle asiatica*

Species	Stomatal Indices: Upper Surface	Stomatal Indices: Lower Surface	Palisade Ratios
Herpestis monniera	11.5–13.6–15.9	14.9–16.3–17.7	1.25–1.75–2.25
Hydrocotyle asiatica	11.6–15.2–20.5	15.9–18.9–21.1	3.25–4.00–5.75

DISCUSSION

Herpestis monniera and *Hydrocotyle asiatica* belong to two different families. Accordingly they differ to a great extent in their anatomical features. In their habitat too these vary a great deal; *Herpestis monniera* grows in more moist places than *Hydrocotyle asiatica*, consequently it possesses such structures as are characteristic of plants growing in moist localities. The presence of air chambers and the absence of collenchyma or sclerenchyma in the stem, inadequate differentiation of mesophyll into palisade and spongy tissues in the leaf, and the reduction of vascular elements both in the stem and the leaf are some of the important characteristics which point out to its habitat which is more of a hydrophytic nature than of the mesophytic one. *Hydrocotyle asiatica*, on the other hand, shows somewhat xerophytic characteristics. The salient differences in their anatomical features which may help one to distinguish these two species are therefore the following:

	Herpestis monniera	*Hydrocotyle asiatica*
	Stem Structure	
1.	Collenchyma is absent.	A well-developed collenchyma is present below the epidermis.
2.	The cortex possesses large air chambers and is thus lacunar.	The cortex has only small intercellular spaces.
3.	The stele is reduced and consists of a continuous ring of a few phloem and xylem elements.	The stele is broken up into 7 vascular bundles arranged in a ring.
4.	Sclerenchyma is absent.	Sclerenchyma surrounds the peripheral side of each vascular bundle.
	Leaf Structure	
5.	Epidermal cells are wavy walled in surface view, with lower surface showing greater waviness.	Epidermal cells are straight walled and polygonal in surface view.
6.	Stomata are present on both the surfaces. There is no definite arrangement of subsidiary cells; usually 3 or more cells surround the stomata. The epidermal cells and stomata are smaller and less in number per sq. mm. leaf area.	Stomata are found on both the surfaces, each surrounded by 3 or more epidermal cells. The number of stomata and of epidermal cells are greater per sq. mm. leaf surface.
7.	Typical glandular hairs on both surfaces.	Hairs are absent.
8.	Cuticle is strongly developed, occurring as prominent striations.	Cuticle is not so well developed, occurring as faint striations.
9.	The mesophyll is poorly differentiated into palisade and spongy layers.	The mesophyll is well differentiated and there are 2–3 layers of palisade cells.
10.	Occasionally a solitary crystal of calcium oxalate may be found in the spongy mesophyll.	Rosettes of calcium oxalate are found in the mesophyll.
11.	Vascular tissue both in the lamina and the midrib is greatly reduced.	Vascular tissue is very much developed.
12.	Collenchyma is absent in the midrib region.	Collenchyma is present below each epidermis.
13.	Values representing stomatal indices and palisade ratios are lower. The range of stomatal indices lies between 11.50 and 17.65; palisade ratios between 1.25 and 2.25.	The stomatal indices and palisade ratios are much higher, the former varying between 11.58 and 21.05 and the latter between 3.25 and 5.75.

SUMMARY

The present investigation deals with the anatomy of the leaf and stem of *Herpestis monniera* and *Hydrocotyle asiatica*, both of which are known as *Brahmi*.

The stem of *Herpestis monniera* shows on the outside a layer of epidermis followed by a wide lacunar cortex. The endodermis surrounds internally a narrow zone of pericycle and a wide continuous ring of vascular tissue. In the center is found a small pith.

Characteristic glandular hairs and stomata are present on both the surfaces of the leaf of *H. monniera*. The epidermal cells are wavy walled. The differentiation of mesophyll into palisade and spongy tissues is poor and the vascular tissue is also much reduced. A distinct bundle sheath surrounds each vascular bundle.

The stem of *Hydrocotyle asiatica* consists of a single layer of epidermis, followed by a narrow ring of collenchyma and a broad region of parenchyma in which are distributed seven vascular bundles in a ring. A distinct endodermis is not to be seen. Each vascular bundle is surrounded toward the periphery by a semilunar patch of sclerenchyma. The vascular bundle is of the open, collateral type. Resin canals are found distributed in the cortex.

The leaf of *H. asiatica* is characterized by a petiole having more or less similar structure as noted in the case of the stem. Stomata are present on both the surfaces, but hairs are absent. The mesophyll is differentiated into 2–3 layers of palisade cells on the ventral side and 5–7 layers of spongy cells on the dorsal side. The midrib has a collenchyma below each epidermis.

These two species differ greatly in their stomatal indices and palisade ratios, *Hydrocotyle asiatica* possessing greater values than *Herpestis monniera*. Other important anatomical differences between the stems and the leaves of these species have also been indicated.

REFERENCES

(1) Sircar, N. N., *Science and Culture*, **6**, 120(1941).
(2) Dymock, W., Warden, C. J. H., and Hooper, D., "Pharmacographia Indica," Vol. 3, London, 1893.
(3) Bose, K., and Bose, N. K., *J. Indian Med. Assoc.*, **1** (1931).
(4) Wali, M. A., and Katti, M. C.-Tummin, *Proc. Ind. Acad. Sci.*, **5A**, 109(1937).
(5) Wallis, T. E., and Dewar, T., *Quart. J. Pharm. Pharmacol.*, **6**, 347(1933).
(6) Salisbury, E. J., *Phil. Trans. Roy. Soc.*, **216**, 1(1927).
(7) Vesque, quoted by Hans Solereder in "Systematic Anatomy of the Dicotyledons" English Ed., Oxford, 1908.

Book Review

Inorganic Chemistry, by W. NORTON JONES. The Blakiston Company, Philadelphia, 1947, xii + 817 pp. + 49 pp. Problems and Questions. 17 x 23.5 cm. Price $4.25.

Just as newer developments in the field of chemistry are adding to and changing previous ideas, the author has endeavvored to revise the older order of presentation of a first year course in college chemistry. This has been done for the purpose of increasing student interest and to enable him to better grasp and retain the facts in the light of modern concepts of electronic structure and the periodic relationship of the elments.

For conservation of time on the part of the student, the treatment of the historical aspects is limited to brief sketches and portraits of those men and to those events which have been so important in shaping the course of the science of chemistry from a cultural and historical standpoint.

The theme followed by the author to maintain interest is a presentation of the theoretical aspects, interspersed with descriptive chapters and practical application.

Following each chapter bibliographies on the particular subject covered are included for the convenience of the student.

This volume should be interesting and valuable to the first year student in college chemistry either as a textbook or as an adjunct to the one in use.—NEULON DEAHL.

Thixotropic Mineral Gels and Their Therapeutic Possibilities*

By HERMAN J. SCHNEIDERWIRTH and PAUL W. WILCOX†

Some aspects of thixotropic behavior are discussed and experimentally developed thixotropic systems in water dispersions of alkaline earth metal salts are described. Factors influencing and producing thixotropy in these systems are discussed. The possible application of thixotropic gels as therapeutic agents is presented.

THE DISPERSION of small solid particles in a fluid sometimes results in the formation of a rigid gel when the mixture is at rest, but upon shaking the gel becomes a readily flowing suspension, called a sol. This transformation takes place under isothermic conditions and often is reversible at will for an unlimited period of time. Thixotropy is the name given to this physical behavior. It is called a "phenomenon" indicating that it is something beyond ordinary conception, something yet unexplained.

Some liquid dispersions of small mineral particles exhibit thixotropy naturally, but it is possible also to produce thixotropy artificially under experimental conditions. However, no definite rules exist to aid a research worker in establishing a given thixotropic system. This is strikingly demonstrated by Pryce-Jones (1) who states: "It may be safely assumed that at the present time no theory can explain the behavior of thixotropic systems or can predict the type of flow resulting from a dispersion of a given volume of fine particles in a fluid."

Several theories have been advanced to explain thixotropic behavior. One of them suggests that attracting and repelling forces of an electric nature acting upon or between the surfaces of the particles rule the phenomenon. These forces, some of which are believed to be London-van der Waals forces, compel the colloidal particles to arrange themselves toward each other in a certain pattern resulting in gel formation. As shown by Schalek and Szegvari (2) and by Hauser (3) in ultramicroscopic studies, the particles first lose their translatory, then their rotatory Brownian movements. Thereafter they are found to be evenly built into a semisolid gel structure and placed motionless face to face or end to end, separated by the dispersing medium. This peaceful arrangement easily is destroyed by agitation. Thereby, the particles are torn loose and slide along or tumble over each other. Everything is temporarily out of line. Brownian movement starts again and a readily flowing sol results. The latter is sustained for seconds or days, depending upon the thixotropic condition present. During this time the gel state gradually is being restored. Unfortunately, convincing experimental evidence of the theory outlined and of others advanced is lacking.

Thixotropy by no means is limited to solid particles of minerals in a fluid. Organic colloids, foams, and emulsions may be made to exhibit thixotropy (4). In short, the latter is a universal colloidal phenomenon, unlimited in scope. Complex, and as yet unexplained functions of living cells are influenced by thixotropic behavior (5). Among others, the appearance of neoplasm in parts of the body remote from the seat of a primary tumor may be due to the ability of cancer cells to exhibit thixotropy during some stages of their migration. A closer study of the thixotropic phenomenon in biocolloids may yield important clues to the understanding of many vital biologic processes. Finally, one can speculate that thixotropic behavior of colloids in an infinitesimally small dynamic way may possibly correlate with the electric theory of matter as applied to molecules and atoms. As the particle size of matter decreases, electric energy acting upon or between the particles

* Received June 7, 1947, from the Department of Pharmaceutical Research, Medical Research Division, Sharp & Dohme, Inc., Glenolden, Pa.

† The authors gratefully acknowledge the work done by Dr. L. D. Wright and Helen R. Skeggs of the Department of Pharmacology, Medical Research Division, Sharp & Dohme, in carrying out the rat experiments. They are also indebted to Mr. Wm. F. Happich, Jr., for painstaking and laborious analytical work on the rat ash residues.

increases until it reaches the tremendous proportions often encountered in subatomic phenomena.

This presentation is limited to experimentally produced thixotropic systems of aqueous dispersions of alkaline earth metal salts and their possible practical applications in pharmacy.

EXPERIMENTAL

Particle Size and Shape.—Past studies of strongly thixotropic systems made by different investigators (6) and (7) show that most favorable conditions are present when the particles have the following characteristics. They should be from submicronic to 5 microns in size, anisometric rather than isometric, and of plate or rod-like shape. We were not able to produce such particles by extensive mechanical treatment of aqueous dispersions of finely powdered mineral salts in a colloid mill. However, when aqueous solutions of alkaline earth metal salts such as calcium, magnesium, barium, and strontium chlorides were allowed to react under specific conditions with alkaline salts of phosphates, carbonates, and silicates, colloidal dispersions of the respective water-insoluble salts that by subsequent treatment could be converted into thixotropic gels were obtained. Particle size and shape were influenced mainly by the temperature at which the reactions took place. Agitation and rate of flow at which the solutions were combined were second in importance. The solutions were prepared in concentrations close to the saturation point at the respective temperatures. Approximately stoichiometric equivalent quantities of the reacting chemicals were used.

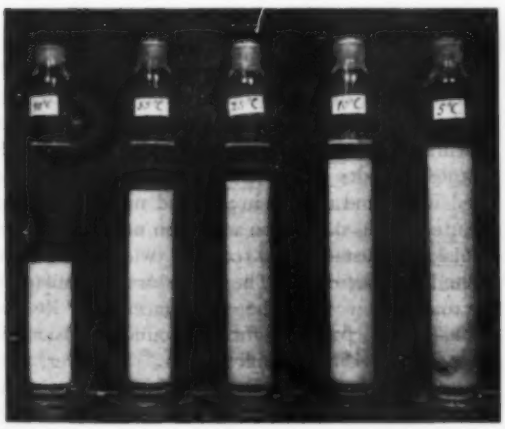


Fig. 1.—Dispersions obtained at 5°, 10°, 25°, 55°, and 90° after standing twenty-four hours at room temperature.

The following is an example, showing the preparation of a mixture of calcium and magnesium phosphates and carbonates at different temperatures and the degrees of dispersion of the particles obtained thereby.

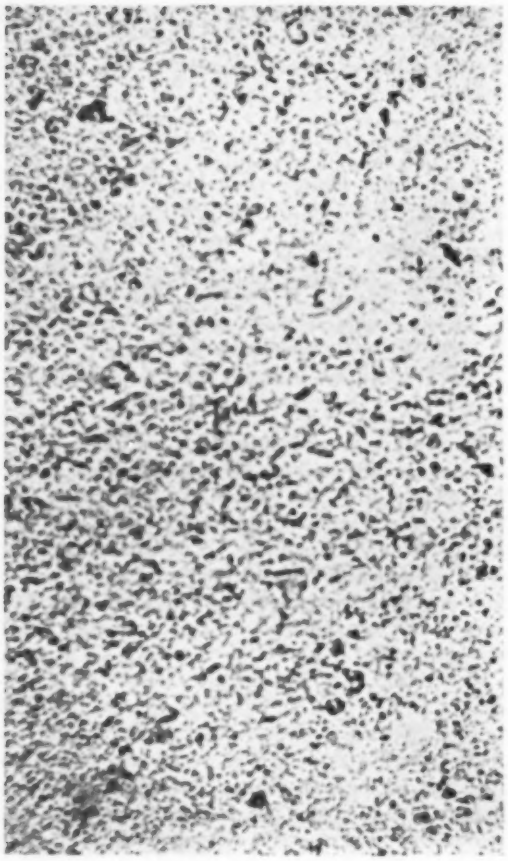
Two solutions were prepared in the following manner:

Solution A		
Calcium chloride, crystalline.....	122.25	Gm.
Magnesium chloride, crystalline..	6.40	Gm.
Distilled water, a sufficient quantity, to make.....	1000	cc.
Solution B		
Sodium phosphate, dibasic, anhydrous.....	108.00	Gm.
Sodium carbonate, monohydrated.	16.25	Gm.
Distilled water, a sufficient quantity, to make.....	2000	cc.

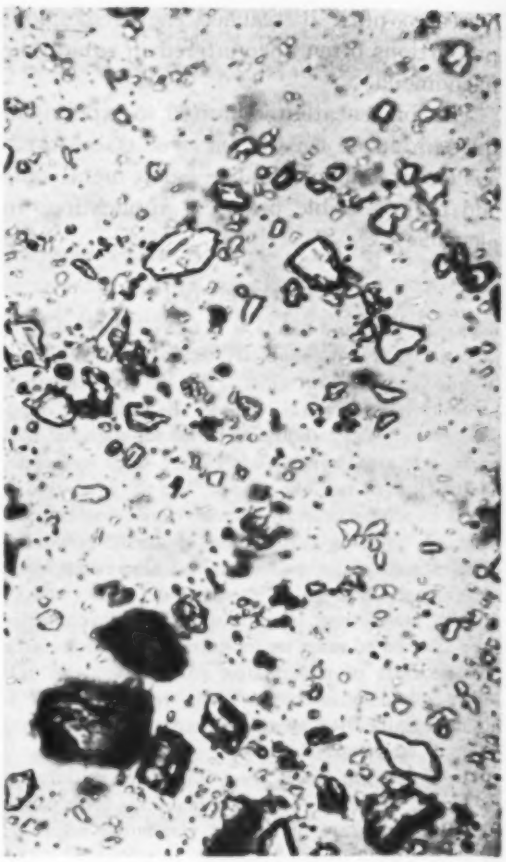
Both solutions were cooled to 5°. One hundred cubic centimeters of solution A was added to 200 cc. of solution B in a slow, even stream while agitating solution B. The voluminous precipitate was shaken for ten minutes and transferred in part to a graduated 100-cc. cylinder. The same procedure was repeated with the remaining solutions A and B after warming both to 10°, 25°, 55°, and 90°. The suspensions obtained under these conditions are shown in Fig. 1.

Conversion into Thixotropic Gels.—The supernatant liquids from the dispersions were decanted to remove the bulk of the water-soluble electrolytes formed during the chemical reaction. Thereafter filtration by suction, using a Büchner funnel, produced soft gels from which additional water was removed in a press. It was comparatively easy to obtain in this manner gels containing from 30% to 35% total solids. Of course, concentration may be regulated at will to meet specific requirements. The mineral gels prepared in this manner formed heavy, semisolid "cakes" that could be broken up and converted into thick pastes by mechanical mixing. Thereafter, sufficient citric acid to give a concentration of 0.5% to 1.5% was added and dissolved by mixing. This resulted in a slight softening of the pasty mass. The *p*H of such mixtures approximated 4.5.

When this *p*H was raised by the addition of an alkali such as sodium hydroxide, sodium carbonate, ammonia water, or an amine to a point above *p*H 5.0, the mixture was converted into a liquid sol that exhibited thixotropy. An increase of *p*H above 6.5 gradually caused the reconversion of the sol into a thick paste with loss of thixotropy. Thus, within a narrow *p*H range (approximately 5 to 6.5) a thixotropic system was established. The time of conversion from the sol to the gel state as well as the degree of viscosity of the sol and the rigidity of the gel varied with different alkaline earth metal salts. In most experiments reconversion took place within a few minutes and the sols were of comparatively low viscosity. They could be poured easily and would pass through a narrow gauge hypodermic needle without difficulty, whereas the subsequently formed gels were rather rigid in structure. It was further found that the *p*H range of thixotropic be-



Thixotropic Gel—415×



Emulsion—415×

Figure 2

havior could be broadened considerably, and thixotropy in general enhanced, when carbohydrates such as lactose and dextrose, or hexahydric alcohols such as sorbitol or mannitol, were added to the alkaline earth mineral gels and dissolved therein. Thus thixotropy was obtained within a *p*H range of from 4 to above 10.

Some Properties of the Thixotropic Mineral Gels.—The size and shape of particles in a thixotropic gel prepared from a dispersion obtained at a temperature of 55°, as described above, are shown in one of the photomicrographs of Fig. 2. As a comparison a photomicrograph of an emulsion that was prepared from an aqueous dispersion of finely powdered mineral salts having substantially the same composition as those present in the thixotropic gel is presented. The emulsion was prepared in the following manner:

Calcium phosphate, dibasic, fine powder	174 Gm.
Calcium carbonate, precipitated, fine powder	16 Gm.
Magnesium phosphate, dibasic, fine powder	5 Gm.
Magnesium carbonate, light, fine powder	5 Gm.
Cod-Liver Oil, U. S. P.	200 cc.
Acacia, fine powder	50 Gm.
Distilled water, *q. s.*	1000 cc.

The acacia powder was added to the cod-liver oil and dispersed by trituration. One hundred cubic centimeters of distilled water was added and the mixture was emulsified. The combined calcium and magnesium salts were mixed with 450 cc. of distilled water and treated in a colloid mill for twenty minutes. This dispersion was then mixed with the emulsion, adjusted to 1000 cc., and twice put through a hand homogenizer. The procedure is similar to the one given in "The Pharmaceutical Recipe Book," ed. 3, page 44, for compound emulsion of magnesium trisilicate and kaolin. However, the product prepared by us was superior on account of the additional colloid mill and homogenizer treatment. Cod-liver oil was used instead of mineral oil in order to prevent a possible interference with calcium absorption in animal tests which are described herein and in which tests this emulsion was used.

The absorption and utilization of calcium from the thixotropic gel in comparison with the emulsion described were determined in preliminary *in vivo* tests on 50 rats in the following manner:

Fifty young albino rats of 85 Gm. average weight were kept on a calcium-free diet for three weeks. Thereafter, one group of 15 rats was fed by stomach tube 1 cc. of a thixotropic gel prepared as described above. Another group of 15 rats was fed 1 cc. of the emulsion described above. After one, two, and five hours, 5 rats of each group were sacrificed and the whole intestinal tracts were dissected and reduced to ash. Four unfed control rats were treated in the same manner.

Calcium Absorption

1. Thixotropic Gel

	Found in Intestine	Absorbed
Intake (per rat)..	64.70 mg.	.....
1 hr.....	63.53 mg.	1.17 mg.
2 hr.....	62.96 mg.	1.74 mg.
5 hr.....	55.24 mg.	9.46 mg.
2. Emulsion		
Intake (per rat)..	57.50 mg.	.....
1 hr.....	59.30 mg.	None
2 hr.....	58.94 mg.	None
5 hr.....	51.28 mg.	6.22 mg.
3. Unfed Controls.	1.05 mg.	.....

Of the remaining 16 rats two groups of 6 rats each were fed daily 1 cc. of the two different products for four successive days. Four rats served as controls. On the fifth day these rats were sacrificed and the intestinal tracts were dissected out and discarded. The animals minus intestinal tracts were reduced to ash and the calcium in the ash residue was determined.

Calcium Utilization

1. Thixotropic gel fed to 6 rats:
   Intake (per rat).....64.70 mg. Ca daily for 4 successive days
   Average found in 6 rats minus intestines.....366.95 mg. Ca—gain 119.10 mg. (corrected for weight)
2. Emulsion fed to 6 rats:
   Average intake.....57.50 mg. Ca daily for 4 successive days
   Average found in 6 rats minus intestines.....325.11 mg. Ca—gain 85.01 mg.
3. Unfed Controls:
   Average found in 4 rats minus intestines.....240.10 mg. Ca.

The figures obtained are statistically significant enough to conclude that the thixotropic gel was definitely better absorbed from the intestinal tract of rats and better utilized by their body than was the emulsion. Since the number of animals in each group was rather small, no attempt is being made to evaluate the advantages of one product over the other in a percentage form. Such figures we hope to be able to present after completion of further animal tests conducted on a larger scale.

DISCUSSION

Since this research was aimed at a possible application of the thixotropic gels as therapeutic agents, a critical evaluation of the practical advantages gained seems to be in order.

Substantially aqueous, fluid medications containing fine, solid particles immiscible with or insoluble in water usually are prepared in the form of suspensions or emulsions. Often there is a separation of the liquid phase upon standing. Sedimentation and coagulation of the particles into a lumpy precipitate impossible to redisperse by shaking is another common occurrence. Further, sometimes the use of large amounts of viscous, gummy or oily substances, needed to obtain permanent suspension, limits the concentration of therapeutically active ingredients. These admixtures also often coat the particles to a degree that may interfere with their absorption and utilization by the body. These products contain an abundance of uneven, sharp-edged, gritty particles whose unpleasant taste can be masked only feebly by the skill of the pharmacist. In such systems the particles are in an uncontrollable, unorganized state. Some of them show Brownian movement, and others simply follow the law of gravity and sink to the bottom.

Thixotropic gels in contrast are controllable, regimented systems. The disadvantages inherent in the comparable forms of application as outlined above largely are overcome. Concentrations of from 30% to 40% of fine solid particles may be incorporated into the semisolid gels, yet the sols pour easily. Such concentrations cannot be obtained in pourable mechanical suspensions or emulsions.

Stability of the thixotropic gels is another important gain. Separation of the liquid phase does not take place except for a slight syneresis common to all gels and caused by shrinkage after standing for several months. Smoothness and creamy consistency, due to fine particle size and to thixotropy itself, is another practical advantage for oral preparations. Finally, evidence for increased absorption and utilization of calcium from a thixotropic gel as compared with an emulsion when both were fed to calcium-starved rats have been shown in the experimental portion of this communication. It is at least possible that similar results can be obtained in human subjects.

To obtain optimum therapeutic response in general mineral deficiencies, additional mineral elements known to be needed by the body, as described in a recent review by Richards (8), were incorporated in some of our experimental lots. Such additions did not interfere with stability or other characteristics of the thixotropic gels.

Another logical step was the addition of vitamins. The writers have prepared many such products and find that fat-soluble vitamins such as vitamin A, vitamin D, and α-tocopherol, as well as the water-soluble vitamins such as ascorbic acid and members of the vitamin B complex, remain evenly dispersed

throughout the thixotropic gels. Losses in vitamin potency after standing for twelve months were moderate and within expected limits. This is understandable when one realizes that the vitamins are in a state of complete rest, imprisoned in a solid gel in the presence of antioxidants which had been added to enhance stability.

SUMMARY

1. Some aspects of the present status of our knowledge of thixotropic behavior are discussed briefly.

2. Experimentally developed thixotropic systems in aqueous dispersions of alkaline earth metal salts are described. The factors influencing and producing thixotropy in these dispersions are particle size and shape, concentration, *p*H and the presence of citrates, carbohydrates, or hexitols.

3. Possible applications of the prepared thixotropic gels in mineral therapy and their use as adsorbents or vehicles for therapeutic agents, are presented. The advantages of such medications are: high concentration of ingredients in a gel-sol form that is reversible at will; stability; freedom from grittiness; controlled, even distribution of ingredients throughout the gels; and possible increase in therapeutic response.

REFERENCES

(1) Pryce-Jones, J., *J. Oil Colour Chemists' Assoc.*, **17** 305(1944).
(2) Schalek, F. E., and Szegvari, A., *Kolloid-Z.*, **32**, 318-19; **33**, 326-34(1923).
(3) Hauser, E. A., *ibid.*, **48**, 57-62(1929).
(4) Whitfield, C. F., *Trans. Faraday Soc.*, **85**, 342(1939).
(5) Alexander, J., "Colloid Chemistry," Reinhold Publishing Corp., New York, 1944, vol. V, p. 872.
(6) Shilling, D. H., and Maslow, H. L., *J. Biol. Chem.*, **78**, 661(1928).
(7) Engelhardt, W., *Kolloid-Z.*, **102**, 217-32(1943).
(8) Richards, W. G., *J. Lancet*, **65**, 315-21(1945).

Iodine Research Award Nominations Requested

Nominations are now being received by the AMERICAN PHARMACEUTICAL ASSOCIATION for the Iodine Educational Bureau Award recognizing outstanding research in the chemistry and pharmacy of iodine and its compounds as applied in pharmacy or medicine. Any member of the ASSOCIATION may propose a nominee by submitting specific identification of the work to be considered in the competition, a biographical sketch of the nominee including date of birth, and a list of his publications. Eight copies of the nomination must be submitted to the Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION, Dr. Robert P. Fischelis, 2215 Constitution Ave., N. W., Washington 7, D. C. To be eligible for the 1948 Award, nominations must be received before January 1.

Establishment of the new iodine award was announced at the Milwaukee convention of the AMERICAN PHARMACEUTICAL ASSOCIATION, which will administer the competition.

A nominee must be a resident of the United States or Canada. He must have accomplished outstanding research in the chemistry or pharmacy of iodine and its compounds as applied in pharmacy or medicine.

During the period covered by the nomination the nominee shall have been actively engaged in, shall have completed, or shall have published a report upon the line of investigation for which the award is made. During a period of two years prior to the date of nomination, the nominee shall not have been engaged in research under the sponsorship of the Iodine Educational Bureau, Inc.

The award consists of $1000 and a diploma setting forth the reasons for selection of the recipient. It may be presented biennially at the annual meeting of the AMERICAN PHARMACEUTICAL ASSOCIATION.

The recipient will deliver a paper or lecture upon the subject of his scientific work at the meeting at which the award is conferred. His paper, or address, will then be published in the JOURNAL of the AMERICAN PHARMACEUTICAL ASSOCIATION. In addition to the sum of the award, the recipient will receive an allowance of not more than $250 to defray his expenses in attending the meeting.

The recipient will be selected by an award committee which is appointed by the chairman of the ASSOCIATION's Council and which functions under prescribed rules.

Should the award committee fail to find a suitable recipient in any biennium, two awards may be made during the next biennium in the same or successive years, if suitable recipients are selected.

Lubricants in Compressed Tablet Manufacture*,†,‡,§

By J. EDWARD WOLFF,‖ H. GEORGE DEKAY, and GLENN L. JENKINS

A theoretical discussion of the possible causes for sticking and binding during compression of granulations is presented together with fundamental research on the mode of action of a lubricant. One hundred seventy-one water-soluble compounds were studied for lubricating properties. Two per cent of a compound lubricant composed of equal parts of Dreft, sodium oleate, and Maprofix gave satisfactory lubrication to 72% of the tested granulations.

THE MANUFACTURE of tablets, as we know them today, has recently had its one hundredth anniversary. There has been little change in the list of lubricants appearing throughout this period and today we still find talc, the stearates, mineral oil, and boric acid commonly employed.

A lubricant is generally defined as a substance which is added to the granulation to insure uniform feeding into the die and to prevent the material from adhering to the punches and die during and after compression.

The exact manner in which a lubricant accomplishes these important functions is not clearly understood. It is known that a compound may offer lubrication to one granulation, while it fails to lubricate another. For some substances no lubricant is required, although generally it is necessary to add one before compression. The amount of lubricant used will vary, and be dependent upon both the granulation and the lubricant selected.

The questions arising are: Why do some substances require a lubricant, and why does a lubricant appear to show specificity in action?

This paper attempts to disclose some theoretical material published in allied fields of work which may in time bring forth answers to our questions. The thesis is divided into two portions, Part I being devoted to exploring the mode of action of a lubricant and Part II consisting of a study of water-soluble compounds for lubricating properties.

* Received June 26, 1947, from Purdue University, School of Pharmacy, Lafayette, Ind.

† Presented at the meeting of the American Association for the Advancement of Science, Pharmacy Subsection, Boston, Mass., Dec. 28, 1946.

‡ Abstracted from a thesis presented by J. Edward Wolff to the Graduate Faculty of Purdue University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Sept., 1945.

§ This work was made possible through the research grant of Sharp and Dohme, Inc., Philadelphia, Pa.

‖ Present address: Medical Research Division of Sharp and Dohme, Inc., Glenolden, Pa.

THEORETICAL DISCUSSION

Rodwell (1) reports that the lack of sufficient lubricant will result in enough friction to cause the tablet to crack. Husa (2) states that more lubricant is needed where there are excessive fines since fine powder has a greater tendency to stick to the face of the punch.

Various physical and chemical factors may enter into the causes for picking, sticking, and binding during compression. Peddie (3) points out that many substances have their melting points lowered with an increase in pressure. The migration of alkaloids to the surface of a tablet during compression suggests some electrical phenomena. Richards, *et al.* (4) state that volatile substances are more compressible than nonvolatile, as the nonvolatile compounds are already under internal pressure. Richards and Mathews (5) conclude that compressibility is large when the surface tension is small, and that the density is greater in compounds with a large surface tension and a small compressibility.

The conversion of sodium bicarbonate to sodium carbonate under pressure has been reported (6). Spring (7) states that bodies remain solid under compression unless they occupy a smaller volume in the liquid than in the solid state. Hallock (8) asserts that the melting point and liquidity of a product are as important as its constituents in determining the possibility of a chemical reaction under pressure. Johnston and Adams (9) observed three layers in a compressed body: the vitreous outer surface, suggesting the flow of a viscous liquid under pressure, a granular layer directly beneath the vitreous surface, and a matted crystalline layer within.

Creelman and Crocket (10) refer to the work of Tait at the University of Edinburgh where his special galvanometer recorded that the fall in temperature on relieving pressure is considerably less than the rise on the application of pressure.

Richards (11) made studies on electrification by impact whereby frictional electricity was compensated. The charge produced was directly proportional to the velocity of the piston and there was no immediate limit to the charge for most solid dielectrics.

EXPERIMENTAL

Part I

Experiment 1.—To determine the most effective way of incorporating the lubricant, fourteen water-soluble compounds were selected. Each was divided into three parts. The first portion was screened through a 120-mesh sieve and dusted on the granulation. The second portion was dissolved in water to make a nearly saturated solution and this solution was incorporated with the granulation by tumbling. The third portion was likewise dissolved in water and the solution was sprayed on the granulation. In the second and third portions the granulation was air dried for three days prior to compression. Aspirin-Starch Granulation (Dow) was the test granulation. The lubricants applied in solution form were found to be the most effective.

Experiment 2.—Sixteen granulations were studied for general structural arrangement under a hand lens magnifying 14 diameters and a compound lens magnifying 180 diameters. Compression studies with the Colton single punch machine revealed that there was no correlation between structure and compressibility of the screened granulations, as granules composed of masses of rectangular crystals were just as compressible as those composed of cubical crystals, or those having no common structural arrangement. In all tests, those granules having numerous interstices were most compressible.

Relative potential studies, using a properly grounded electroscope, showed that the colored boric acid carrying a positive charge separated in only 32% of the tests when the granulation carried a negative charge, whereas 75% of the tests showed separation when the granulation carried a positive charge. Compression studies using the Colton single punch and the Stokes RB-2 rotary machines revealed that the degree of adherence of the lubricant to the granules does not appear to have any influence on the ability to lubricate the granules during compression, and screening out the free colored lubricant did not alter compression.

Experiment 3.—The fact that crystalline substances, having no center of symmetry, show piezoelectric properties and are capable of producing electric charges when they are compressed, led to the opinion that possibly, during the rapid compression of some compounds, a charge, too large to be carried off by the grounded machine, is built up. A lubricant might act as a conductor by providing more points of contact or it might act as an insulator. Resistance to the passage of a current creates heat, heat causes substances to melt, and the melting points of some substances are lowered under pressure. The adhesive nature of most granulations would be likely to cause binding or sticking, should sufficient heat be generated.

A device was made (Fig. 1) whereby a compound could be placed in an insulated cylinder, pressure

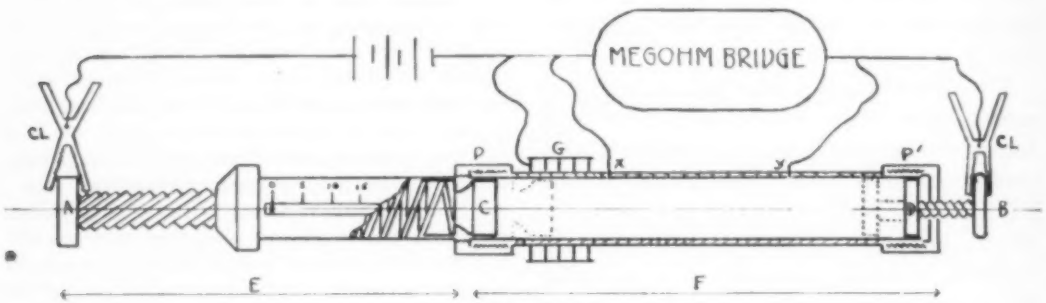


Figure 1

Key:
E—Monsanto hardness tester
F—Bakelite cylinder
G—Soft lead guard and ground
P and *P'*—Metal caps
CL—Clamp

It was impossible to observe the appearance of a lubricant on a granulation when both were white. Boric acid was selected as the lubricant. It was colored with a 10% solution of Amaranth, dried, pulverized, and sifted through a 120-mesh sieve. The colored lubricant was added to the granulation and thoroughly mixed by tumbling.

Microscopic study revealed that the lubricant becomes lodged in the roughened cavities of the granules and does not appear to envelop the granules (12). Although nearly all of the granules possessed rough edges, in 56% of the samples the lubricant did not adhere and a considerable amount of colored lubricant remained on paper after thorough mixing.

applied, and the resistance to the passage of a current measured on a Megohm Bridge (Type 544-B No. 99 General Radio Corporation).

All compounds were introduced so that packing was as near uniform as possible throughout the tube *F*. Pressure was applied to the brass piston *C* until 15 Kg. was recorded. Points *x* and *y* were exactly 8.2 cm. apart and the diameter was 2.54 cm. A current of 120 volts was introduced and the resistance between points *x* and *y* read directly on the Megohm Bridge. The apparatus was grounded through the Megohm Bridge.

Wet granulations were made from which samples were removed at various stages of drying. Each

sample was studied for compressibility and moisture content and specific resistivity. The resistivity increased considerably as the moisture content decreased. None of the nonlubricated granulations was compressible with a moisture content greater than 4%. Each of the dry granulations offered a resistance of over 50,000 megohms to the passage of a current of 120 volts.

The resistivity to conductivity was determined on thirty-six dried and powdered compounds suggested as lubricants. In all but eight the resistivity was high. Those offering the least resistance to the passage of the current were: bentonite, magnesium trisilicate, Margel, Arctic Syntex M, magnesium silicate, sodium borate, Kelgin, and Tegin.

The action of a conductor such as bentonite on a granulation which offered a resistance of over 50,000 megohms was studied under a pressure of 15 Kg. From 1% to 5% bentonite was employed in several tests and in all tests the resistance did not change. When a static charge of 300 volts was placed on the compressed material, however, the charge fell off rapidly whereas a static charge placed on the compressed nonlubricated granulation remained with only a gradual loss of charge.

Experiment 4.—Beery (13) states that the lubricant plays an important part in the disintegration of a tablet.

To show this effect, the standard Aspirin-Starch Granulation (Dow) was prepared by screening through a number 16 mesh sieve, rejecting the fines passing through a number 30 sieve. Thirty-six compounds were selected as lubricants representing wetting agents, soaps, chemicals, and food products. Each was screened through a number 120 sieve, mixed thoroughly with the granulation, and compressed. All granulations were compressed with the Colton 2B single punch machine. Each tablet weighed 6 gr. Ten tablets had an average hardness of 3.5 Kg. when tested.

The food products representing flours and starches caused the tablets to disintegrate most rapidly followed by the wetting agents, chemicals, and finally the soaps. It must be noted, however, that the food products were employed in the largest amounts, followed by wetting agents, soaps, and finally chemicals.

Part II

Experiment 1.—The need for a water-soluble lubricant in the manufacture of compressed tablets has long been recognized, particularly if it is intended that the tablet give a clear solution when dissolved in water.

One hundred seventy-one water-soluble compounds were selected from organic and inorganic compounds and tested for lubricating properties. Solid substances were sifted through a number 120 sieve and incorporated with the granulation by tumbling. Liquids were added and mixed with the granulation by tumbling. One per cent of the lubricating compound was used, because it was found that 1% magnesium stearate offered satisfactory lubrication to the standard granulations, and the smallest possible effective amount of lubricant was desired. The standard granulations were: (*a*) aspirin compound, (*b*) calcium lactate, (*c*) vitamin C, and (*d*) dextrose.

Each granulation was made in sufficient quantity to carry through the entire experimentation and kept in closed containers. The density and moisture content of the granulations were determined at frequent intervals and were found to remain constant. Each granulation was screened prior to lubrication. The initial compression studies were made with the Colton 2B machine using a $^{7}/_{16}$-inch die and curved punches. Compression immediately followed lubrication.

The die and lower punch were removed, examined, washed, and dried before the next sample was compressed. Those compounds offering satisfactory lubrication were then incorporated with the respective granulation and compressed with the Stokes RB-2 rotary machine. In practically every test more lubricant was required when using the rotary than when using the single punch tablet machine although the granulations, the size of the die, and the tablet hardness were the same.

Combinations of all the satisfactory lubricating compounds were made and tried on the standard granulations, and samples of the lubricated granules were compressed using both the single and rotary punch machines. A compound composed of equal parts of Dreft (Procter and Gamble), sodium oleate, and Maprofix (Onyx Oil and Chemical Company) gave good results when 2% of lubricant was employed with twenty-nine different granulations which included twelve commercial samples.

SUMMARY AND CONCLUSIONS

A theoretical discussion of the possible causes for sticking and binding during the compression of granulations is presented together with fundamental research on the mode of action of a lubricant. One hundred seventy-one water-soluble compounds were studied for lubricating properties. Two per cent of a compound lubricant composed of equal parts of Dreft, sodium oleate, and Maprofix gave satisfactory lubrication to 72 per cent of the granulations studied.

The conclusions which may be drawn from this initial work include:

1. Most of the lubricants employed in tablet manufacture are insoluble in water and appear to be more effective than water-soluble lubricants.

2. A lubricant does not envelop a granule but becomes lodged in the roughened cavities of the surface.

3. A lubricant may play a part in the conductance of an electric charge produced during compression as a static charge passed off more readily from some of the lubricated granulations than from others and much more rapidly from a lubricated than from a nonlubricated granulation.

4. A lubricant applied in solution form is more effective than a powder dusted on the granulations.

5. The type of lubricant plays a part in the disintegration of a tablet since food products generally caused tablets to disintegrate more rapidly than wetting agents, soaps, or chemicals.

6. The compound lubricant composed of equal parts of Dreft, sodium oleate, and Maprofix offered satisfactory lubrication for 72 per cent of the tested granulations.

REFERENCES

(1) Rodwell, H., *Pharm. J.*, **75,** 826(1905).
(2) Husa, W. J., "Pharmaceutical Dispensing," ed. 2, Husa Bros., Iowa City, Iowa, 1941, p. 93.
(3) Peddie, W., *Proc. Roy. Soc. Edinburgh*, **13,** 155(1885).
(4) Richards, T. W., Stull, W. N., Brink, F. N., and Bonnet, F., *J. Am. Chem. Soc.*, **31,** 154(1909).
(5) Richards, T. W., and Mathews, J. H., *ibid.*, **30,** 11 (1908).
(6) Anon., *Proc. Am. Drug Mfrs. Assoc., 14th Ann. Meeting*, 1925, p. 183.
(7) Spring, W., *Am. J. Sci.*, **36,** 286(1888).
(8) Hallock, W., *ibid.*, p. 59.
(9) Johnston, J., and Adams, L. H., *J. Am. Chem. Soc.*, **34,** 563(1912).
(10) Creelman, H. G., and Crocket, J., *Proc. Roy. Soc. Edinburgh*, **13,** 311(1885).
(11) Richards, H. F., *Phys. Rev.*, **16** (2), 290(1920).
(12) Anon., *Pharm. J.*, **68,** 46(1902).
(13) Beery, H., *ibid.*, **143,** 174(1939).

United States Pharmacopœial Convention

Seventh Annual Financial Report, Covering the Period from May 1, 1946, to April 30, 1947, and Based Upon the Report of the Auditor

STATEMENT OF INCOME AND EXPENSE FOR THE YEAR ENDED APRIL 30, 1947

Income			
Sale of Pharmacopœias:			
Collections		$40,558.50	
Less: Decrease in Accounts Receivable			
April 30, 1946	$ 5,411.00		
April 30, 1947	890.75	4,520.25	$36,038.25
Sale of Reference Standards, A. M. A. Articles, etc.:			
Collections		$ 6,402.45	
Less: Decrease in Accounts Receivable			
April 30, 1946	$ 916.50		
April 30, 1947	803.00	113.50	
		$ 6,288.95	
Less: Decrease in Collections Pending Remittance to the Treasurer			
April 30, 1946	$ 719.25		
April 30, 1947	487.75	231.50	6,057.45
Interest on Investments:			
Collections		$ 2,630.74	
Less: Amortization of Bond Premiums		80.05	2,550.69
Miscellaneous Income:			
Collections			411.79
Total Income			$45,058.18

Less:				
Expenses				
Publication and Sales Expense			$14,930.30	
Less: Increase in Inventory				
April 30, 1947	$6,475.99			
April 30, 1946	4,014.59		2,461.40	$12,468.90
Administration				4,592.72
Revision			$33,646.16	
Less: Increase in Inventory Reference Standards				
April 30, 1947	$6,033.75			
April 30, 1946	2,073.02		3,960.73	29,685.43
Research				11,474.58
Stationery and Supplies—Inventory Adjustment			(Minus)	85.38
Provision for Depreciation:				
Building			344.91	
Furnishings and Equipment			302.06	646.97
				58,783.22
Excess of Expense Over Income For the Year Ended April 30, 1947				$13,725.04

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS (GENERAL ACCOUNT) FOR THE YEAR ENDED APRIL 30, 1947

Cash on Deposit, April 30, 1946				$62,414.24
Add:				
Receipts				
Sale of Pharmacopœias			$40,558.50	
Sale of Reference Standards			6,402.45	
Interest on Investments			2,630.74	
Use of Text by Others			10.00	
Miscellaneous			401.79	50,003.48
				$112,417.72

Less Disbursements	Publications and Sales	Administration	Revision	Research	Total
Printing and Binding	$14,930.30				$14,930.30
Salaries and Honoraria		$1,700.00	$13,500.00		15,200.00
Clerical		1,148.80	7,998.50	$ 129.90	9,277.20
Meetings		583.42	1,207.13	3,443.29	5,233.84
Supplies		58.00	1,343.24	785.32	2,186.56
Postage and Telegrams		85.20	1,043.86	31.36	1,160.42
Compensation				7,070.59	7,070.59
Headquarters			1,289.95		1,289.95
General		1,017.30	7,263.48	14.12	8,294.90
	$14,930.30	$4,592.72	$33,646.16	$11,474.58	$64,643.76

Add:			
Purchase of Furnishings and Equipment		$ 9.90	
Building Improvements		117.50	$64,771.16
Cash on Deposit, April 30, 1947			$47,646.56

A MORE DETAILED AND SUPPLEMENTARY STATEMENT OF THE REVISION AND RESEARCH EXPENSES

REVISION—MEETINGS

Chairman's Conferences—Transportation, Hotel, Meals, Etc., for the Revision Chairman and Others, for Meetings and Conferences Related to or Required by the Revision Program and Called by the Chairman	$ 1,207.13

REVISION—SUPPLIES

Stationery	$ 275.75
Mimeograph	594.99
Miscellaneous	472.50
	$ 1,343.24

REVISION—SALARIES

Revision Chairman, E. Fullerton Cook	$ 9,000.00
Executive Assistant, Adley B. Nichols	4,500.00
	$13,500.00

REVISION—GENERAL

Reference Standards	$ 3,320.75
A. M. A. Convention Exhibit	625.96
Inter Society Color Council	25.00
American Pharmaceutical Association—1945 and 1946	2,000.00
Receiver of Taxes, Philadelphia	1,180.15
Miscellaneous	111.62
	$ 7,263.48

HEADQUARTERS

Janitor's Salary (One-half)	$ 795.00
Utilities	365.90
Maintenance—Supplies	53.16
Repairs	29.70
Furniture and Equipment	26.19
Insurance	20.00
	$ 1,289.95

RESEARCH

Subcommittees	Meetings, Supplies, Postage, Clerical Travel and General	Technical Assistance	
1. Scope		Assistant to Dr. Youngken for Study on Rhubarb	$ 70.59
Meetings	$2,239.46	Assistant to Dr. Krantz	2,500.00
Miscellaneous	10.20	Assistant to Dr. Beal	4,500.00
5. Botany & Pharmacognosy	28.50		
7. Inorganic Chemicals	300.44		
8. Organic Chemicals	500.00		
13. Ointments, Etc.	466.46		
Amino Acids Committee	489.07		
Anti-anemia Board	121.56		
Sterile Products Board	58.23		
Vitamin Board	190.07		$ 7,070.59
	$4,403.99		4,403.99
Total			$11,474.58

Notes

Notes on the Role of Hydrogen Ion Concentration and Buffer Systems in the Preparation of Ophthalmic Solutions*

By HARRY W. HIND, FRANK M. GOYAN, and T. W. SCHWARZ

THE PAPER entitled, "A New Concept of the Role of Hydrogen Ion Concentration and Buffer Systems in the Preparation of Ophthalmic Solutions," by Hind and Goyan (1) has created sufficient interest to show the need for further clarification of at least two points.

The first has to do with the choice of preservative, especially in connection with "Solution 3 for Preparation of Eserine Solutions" (1). When this solution is used, a salicylate-benzalkonium incompatibility[1] similar to the nitrate-zephiran incompatibility reported by Huyck and Davy (2) cannot be avoided except by choosing some salt of eserine other than the salicylate. A more generally useful solution for eserine can be made by substituting phenylmercuric nitrate (1:100,000) for benzalkonium chloride (1:50,000). A further improvement results from substituting sodium sulfite for sodium metabisulfite as the antioxidant. The slow oxidation of the bisulfite by oxygen of the air causes a decrease in pH which promotes the formation of turbidity upon the addition of eserine salts to old solutions. Although no solution containing sulfite or bisulfite should be kept indefinitely, the substitution of sodium sulfite eliminates some of the undesirable effects of aging. The following formula embodies the changes suggested above.

SOLUTION FOR THE PREPARATION OF ESERINE SOLUTION

Boric acid, C. P. (22.0 Gm./L.)	0.356 *M*
Sodium sulfite (Na_2SO_3)	0.1%
Phenylmercuric nitrate	1:100,000
Distilled water	*q. s.*

The second matter that requires clarification has to do with the ambiguous column heading, "Grams of Sodium Chloride per 100 Cc. to Render Isotonic." In Table V (1) values tabulated under this heading for the Sørensen phosphate buffer system represent the calculated osmotic equivalent of each buffer mixture, expressed as grams of sodium chloride per 100 cc. of water. These values were calculated from the equation,

$$W = 17(L_1m_1 + L_2m_2),$$

which was derived by assuming that contributions to the freezing-point lowering from different solutes in a solution isotonic with blood serum are approximately additive (i.e., $\Delta T_f = L_1m_1 + L_2m_2 = 3.44\ W/58.45$). In these equations, ΔT_f represents the freezing-point lowering of a solution containing two solutes having *molal* freezing-point lowerings of L_1 and L_2, and molal concentrations of m_1 and m_2, respectively. This solution is isotonic with a solution of sodium chloride ($m = W/58.45$; $L = 3.44$) containing W Gm. of salt per 1000 Gm. of water. Values of L_1 and L_2 for different substances, and the significance of these values, are given by Goyan, Enright, and Wells (3). For the Sørensen Buffer mixture containing 90 cc. of $^1/_{15}M$ NaH_2PO_4 and 10 cc. of $^1/_{15}M$ Na_2HPO_4, $m_1 = (0.9/15)$, $m_2 = (0.1/15)$, and L_1 and L_2 have the values 3.22 and 4.22, respectively. The osmotic equivalent tabulated for 100 cc. of this buffer mixture should be one-tenth of the value of W, or 0.38 Gm. of sodium chloride. This is in complete agreement with the value given by Wells (4) and by Hind and Goyan (1), but to clarify this matter in the latter paper, the last column in Table V dealing with the Sørensen phosphate buffer might have been headed: "Osmotic Equivalent of Each Buffer Mixture Expressed as Grams of NaCl per 100 Cc. of Solution. (To render 100 cc. of solution isotonic, add an amount of NaCl equal to the difference between the tabulated value and 0.90 Gm.)"

Conversely, values tabulated for the Palitzsch borate buffer in Table IV (1) under the heading, "Grams of Sodium Chloride per 100 Cc. to Render Isotonic," represent weight of sodium chloride to be added to 100 cc. of the corresponding buffer solutions. These values were calculated by subtracting, from 0.90, one-tenth of the values calculated for W by the use of the equation given above. In view of the complex equilibria involved, the molal freezing-point lowerings for boric acid and for borax were assigned the values 2 and 7, respectively.

In calculations of the type illustrated above, molal and molar concentrations may be used interchangeably, and the computed weights of sodium chloride may be rounded off to one significant figure; greater accuracy is of no therapeutic significance. Other values given by Hind and Goyan may be rounded off to advantage. For example, a 0.2 *M* solution of boric acid, intended either for use with the Atkins and Pantin or the Gifford buffer, is properly made by dissolving 12.4 Gm. of boric acid in distilled water to make 1 L. of solution.

As a matter of possible academic interest, but of

* Received July 1, 1947 from the Barnes-Hind Laboratories, Inc., 430 Post St., San Francisco, and the University of California, College of Pharmacy, San Francisco.

[1] Preliminary experiments show that the boric acid-sulfite solution for eserine, containing any one of several cationic bactericidal quaternary ammonium salts such as Zephiran, Phemerol, and Emulsept, at a dilution of 1:50,000, slowly develops opalescence at room temperature with as little as 0.1% of sodium salicylate. Higher concentrations of sodium or eserine salicylate produce marked turbidity in a few minutes. Bromides and iodides may also form turbid solutions with benzalkonium salts, depending upon concentration. The same solution (without salicylate) can be used to make clear solutions of eserine hydrochloride. Further study is in progress. The need for this study was first brought to our attention by P. G. Devine, U. S. Shea, and W. C. Clark of the Barnes-Hind Laboratories, Inc.

no therapeutic significance, it should be noted that the freezing-point depression calculated for "Buffer Solutions for Group 2 Drugs" (1) is 0.50°, corresponding to an approximately 0.85% solution of sodium chloride. The value of 0.56° corresponds to a 0.95% solution of sodium chloride, a concentration often assumed to be isotonic with blood serum. In the same table, the formula for disodium phosphate is in error by the transposition of a subscript. Another typographical error of minor importance has been noted: the modified Palitzsch buffer is correctly given in Table IV (1), but the buffer range mentioned in the text (12 lines below the table) should have been given as *p*H 7 to 9.

REFERENCES

(1) Hind, H. W., and Goyan, F. M., THIS JOURNAL, 36, 33–41(1947).
(2) Huyck, C. L., and Davy, E. D., THIS JOURNAL, *Pract. Pharm. Ed.*, 8, 27(1947).
(3) Goyan, F. M., Enright, J. M., and Wells, J. M., THIS JOURNAL, 33, 74(1944).
(4) Wells, J. M., THIS JOURNAL, *Pract. Pharm. Ed.*, 5, 99 (1944).

A Note on the Synergism of the Central Nervous System Stimulants*

By RAYMOND P. AHLQUIST

TWO DRUGS with similar pharmacological actions when administered simultaneously often act synergistically. This synergism may take the form of a simple algebraic summation of responses or it may appear as a potentiated response. Most quantitative studies of the synergism of centrally acting drugs have been done using the central depressants. The synergism between the convulsant action of morphine and Metrazol, picrotoxin, nikethamide, and strychnine has been reported by Hazleton and Koppanyi (1).

A knowledge of the type of synergism exhibited by the central stimulants is of practical value when they are used therapeutically as analeptics. In treating barbiturate poisoning, for example, a number of different stimulants may be used in the same patient (2). Since many of the stimulants are relatively long-acting it is possible that the presence of one may significantly influence the action of a subsequent stimulant. This study was undertaken to determine the type of synergism occurring between the central stimulants.

GRADATION OF INTENSITY OF STIMULANT ACTION

RESPONSE		
	A	Little observable effect.
	B	Hyperexcitability and tremors; ears erect; characteristic twitching of nose and ears.
	C	Metrazol and Strychnine: Posture characteristic; forelegs extended; toes twitching; urination frequent. Coramine: Clonic spasm of hind legs only.
	D	Severe clonic spasms of 10 to 30 seconds duration; opisthotonus common with Metrazol and Strychnine.
	E	Severe tonic spasms; death common with Strychnine.

Fig. 1.—Gradation of intensity of stimulant action of metrazol, coramine, and strychnine sulfate when injected intravenously into rabbits.

Metrazol, nikethamide (Coramine), and strychnine sulfate were the stimulants employed.[1] These were chosen because they are commonly used analeptics and because clear, rapid convulsive responses can be obtained on intravenous administration to rabbits. Picrotoxin was not included in this preliminary study because of its delayed convulsive effect which makes it difficult to have its action appear simultaneously with that of another stimulant. No detailed statistical treatment of data was attempted since it was felt that the results obtained were sufficiently clear-cut to render this unnecessary in establishing the general type of synergism present.

METHOD

The stimulant responses to various dosages and combinations of Metrazol, Coramine, and strychnine sulfate were determined in seventy albino rabbits of both sexes and weighing from 1.5 to 2.5 Kg. The drugs were injected rapidly into the marginal ear vein in a volume ranging from 1 to 2 cc. This rapid injection of a small volume was especially important in the case of Coramine since it was found that if larger volumes were injected more slowly the convulsive response was markedly diminished. The stimulant responses were graded as to relative intensity as shown in Fig. 1. The animals were used in groups of ten and no group was used more than four times. Four to ten days were allowed between injections in any one group. In two groups the response to the same dose of Metrazol was tested before and after the series of injections without any significant change in response being noted. All doses are stated on a mg. per Kg. basis.

RESULTS

The dose that would produce type D or E responses in about 50% of the animals tested was first determined for each drug. This dose will be referred

* Received July 31, 1947, from the Department of Pharmacology, University of Georgia School of Medicine, Augusta. Presented to the Scientific Section, A. PH. A., Milwaukee meeting, August, 1947.

[1] The Metrazol and Coramine were kindly supplied by the Bilhuber-Knoll Corp. and Ciba Pharmaceutical Products, Inc., respectively.

to as the CD_{50}. Figure 2 shows the results obtained using increasing doses of Metrazol. It will be seen that the CD_{50} for Metrazol is about 10 mg. The minimum effective dose (MED) was set as the dose that would produce type C, D, or E responses in one or two animals out of a group of ten. For Metrazol the MED was about 7 mg. The CD_{50} for Coramine was found to be about 50 mg. and the MED about 35 mg. For strychnine sulfate the CD_{50} was found to be about 0.18 mg. and the MED about 0.1 mg. These results agree reasonably well with those reported by Hazleton and Koppanyi (1).

Since innumerable combinations of doses of these three drugs could be used it was necessary to limit this study to a determination of the CD_{50} of combinations when given in equal ratio to their respective CD_{50}'s. Two combinations were tested: Metrazol-Coramine and Metrazol-strychnine sulfate. Starting with a mixture of 5 mg. of Metrazol and 25 mg. of Coramine (50% of each CD_{50}) the doses were increased as shown in Fig. 3. It was found that a mixture of 7 mg. of Metrazol and 35 mg. of Coramine (70% of each CD_{50}) was the CD_{50} for this combination. It was also noted that the convulsions produced by this combination resembled those produced by Metrazol alone rather than those of Coramine.

Treating the combination of Metrazol and strychnine sulfate in the same way it was found that a mixture of 6 mg. of Metrazol and 0.1 mg. of strychnine sulfate (60% of each CD_{50}) produced type D or E responses in about 50% of the animals tested. These convulsions resembled those produced by strychnine sulfate alone.

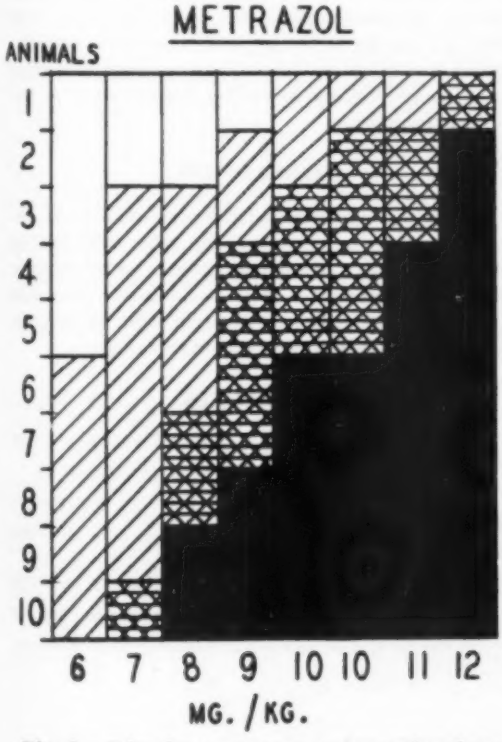


Fig. 2.—Stimulant responses to increasing doses of Metrazol. Gradation of response as shown in Fig. 1.

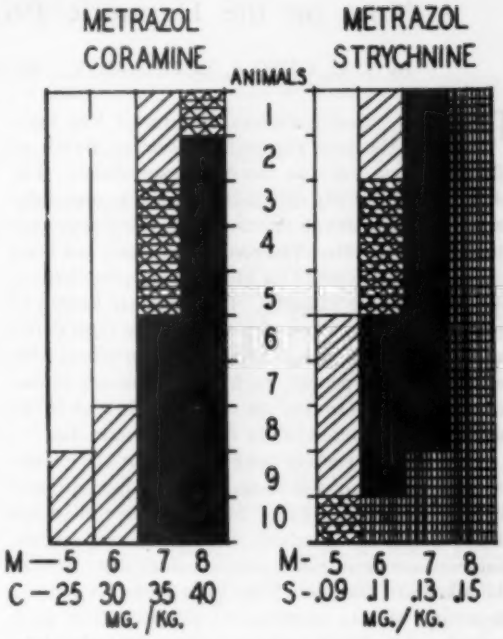


Fig. 3.—Stimulant responses to combinations of Metrazol and Coramine, and Metrazol and strychnine sulfate. Gradation of response as shown in Fig. 1.

DISCUSSION

The type of synergism occurring between these central stimulants is of the deficient additive type when tested on their convulsant actions. The deficiency is probably due to differences in points of action of these substances to produce the convulsions. For example, strychnine acts primarily on the spinal cord whereas Metrazol acts only partially on this structure. Therefore only part of the convulsive effect of the Metrazol would add to the convulsive effect of the strychnine. Another interesting point is that in either combined CD_{50} one of the drugs at least is present in a dosage equal to its MED. This again tends to show that the synergism is of the additive type rather than the potentiated type.

Experimental work should be undertaken to study the synergism of the stimulants in the presence of a depressant. It may well be that a different type of synergism exists between the respiratory stimulating effects or the awakening effects of these analeptics.

SUMMARY

There is an additive synergism between the convulsant effects of the central stimulants Metrazol, Coramine, and strychnine sulfate when tested in rabbits.

REFERENCES

(1) Hazleton, L. W., and Koppanyi, T., *Anesthesiology*, **2**, 427(1941).
(2) Billow, B. W., *J. Lab. Clin. Med.*, **29**, 265(1944).

A Note on the Hypnotic Principle of *Rauwolfia serpentina**

By J. C. GUPTA, SUDHAMOY GHOSH, A. T. DUTTA, and B. S. KAHALI

IN RECENT years considerable interest has been taken in the drug *Rauwolfia serpentina* Benth ex Kurz belonging to the family Apocynaceae. The plant is fairly widely distributed in India, especially in the sub-Himalayan tracts and in the plains near the foot of the hills. The root of the plant has been used as a home remedy for inducing sleep in children and as a cure for insanity. Instances are known to us in which some types of insanity have been cured by the use of 40 doses of the root powder mixed with powdered black pepper, each dose consisting of one tola (about 11.6 Gm.) of the root and 19 corns of the pepper, administered orally every alternate day.

A number of workers have carried out the chemical investigation of this drug. In India Siddiqui and Siddiqui (1) have isolated five crystalline alkaloids three of which—ajmaline, serpentine, and serpentinine—have been tested pharmacologically. Besides the alkaloids, the roots have been found to contain oleoresins, sterols, unsaturated alcohols, oleic acid, fumaric acid, glucose, sucrose, an oxymethylanthraquinone derivative, a fluorescent substance, and mineral salts (2–10).

The presence of 1.21% to 1.36% of alkaloids in the root naturally diverted the attention of the earlier workers to the alkaloids, which were considered as the only active principles responsible for the hypotensive and hypnotic actions. A standardized alcoholic extract containing about 0.5% of alkaloids was therefore recommended by us for clinical trials, and this extract or tincture manufactured by different commercial firms is used by the medical profession in India for the treatment of hyperpiesis and maniacal types of insanity.

Experiments on frogs showed that the ajmaline group of alkaloids acts as a general depressant to the heart, respiration, and nerves, and the serpentine group of alkaloids paralyzes the respiration and depresses the nerves, but stimulates the heart. Experiments on higher animals showed that both ajmaline and serpentine have a depressant effect on the central nervous system and produce a fall of blood pressure whereas serpentinine causes a rise. None of the alkaloids possessed any marked sedative action whereas the alcoholic extract possessed sedative or hypnotic properties, producing drowsiness, narcosis, and fall of rectal temperature. These observations led us to look for the active principle responsible for the sedative action in the nonalkaloidal components of the drug, and our attention was specially focused on the oleoresin.

* Received Feb. 10, 1947, from the Department of Pharmacology and Chemistry, Calcutta School of Tropical Medicine, Calcutta, India.

EXPERIMENTAL

The alcohol-soluble extractives of samples from different sources varied widely, e.g., Bengal 4.32, Behar 5.01, Dehra Dun 7.50, and Assam 12.68%. Aqueous extracts of these alcohol-soluble extractives removed all the alkaloids, and the oleoresins left behind were as follows: Bengal 1.14%, Behar 1.25%, Dehra Dun 1.59% and Assam 1.88%. The oleoresins were carefully dried and extracted thoroughly with petroleum ether (b. p. 35–60°) to remove the oily fraction, which when tested pharmacologically proved to have some irritant properties but no hypnotic action. The resin left behind was extracted with 95% alcohol, the soluble portion being found to be active while the insoluble portion was quite inactive. This purified resin, completely freed from alkaloids, water-soluble constituents, and oils, produced sedative and hypnotic effects in experimental animals such as cats, rabbits, guinea pigs, rats, and frogs. The sedative action commences about three to four hours after administration and persists for more than twenty-four hours after administration. It does not cause a fall of blood pressure but stimulates the muscle of the intestine and uterus. This action is produced immediately. The effective dose of the resin for cat and rats was 1 mg. per Kg. of body weight. The present pharmacological and chemical study was confined mainly to the purified total resin, and the action was found to differ quantitatively according to its source. Work on further fractionation of the resin is in progress.

CONCLUSION

The basic importance of the present findings appears to be the fact that no resin other than that of Hemp has been recorded to possess such hypnotic or sedative properties, and an intensive chemical and pharmacological investigation will, we believe, prove to be a very fruitful line of research.

REFERENCES

(1) Siddiqui and Siddiqui, *J. Indian Chem. Soc.*, **8**, 667 (1931).
(2) Sen and Bose, *Ind. Med. World*, **2**, 194(1931).
(3) Roy, *Patna J. Med.*, Oct., 1931.
(4) Van Itallie and Steenhauer, *Arch. Pharm.*, **270**, 313 (1932).
(5) Chopra, Gupta, and Mukerji, *Indian J. Med. Research*, **21**, 261(1933).
(6) Chopra and Chakravarty, *ibid.*, **29**, 763(1941).
(7) Chopra, Gupta, Bose, and Chopra, *ibid.*, **31**, 71(1943).
(8) Gupta and Kahali, *ibid.*, p. 215.
(9) Gupta, Deb, and Kahali, *Indian Med. Gas.*, **78**, 547 (1943).
(10) Gupta, Kahali, and Dutta, *Indian J. Med. Research* **32**, 183(1944).

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

Published in Two Monthly Editions under Supervision of the Committee on Publications with the Approval of the Council

Committee on Publications

GEORGE D. BEAL, *Chairman*

B. V. CHRISTENSEN — ROBERT P. FISCHELIS

ROY B. COOK — HUGO H. SCHAEFER

SCIENTIFIC EDITION

Editor

JUSTIN L. POWERS

INDEX TO VOLUME XXXVI

INDEX TO AUTHORS

INDEX TO SUBJECTS

JANUARY TO DECEMBER, 1947

AUTHOR INDEX

A

Acker, M., *see* Adams, J.
Adams, J., Acker, M., and Frediani, H. A., iodimetric titrimetric determination of ascorbic acid, 170
Ahlquist, R. P., a note on the synergism of the central nervous system stimulants, 414
Allmark, M. G., *see* Bachinski, W. M.

B

Bachinski, W. M., and Allmark, M. G., on the oxytocic activity of pitocin and pitressin on isolated segments of mammalian uteri by a graded response method, 73
Banerjee, R. P., *see* Basu, U. P.; Mukherjee, S.
Bang, H., synthesis of iodosulfophenylazo and iodocarboxyphenylazo derivatives of 1-amino-8-naphthol-2,4-disulfonic acid, 76
Barnard, R. D., the effect of saccharin ingestion on blood coagulation and the *in vitro* anticoagulant effect of saccharin and of ferriheme, 225; a simple color reaction for piperazine, 224; *see* Mackowiak, E. J.
Basu, N. K., and Dandiya, P. C., chemical investigation of *Premna integrifolia* Linn., 389
Basu, U. P., Mukherjee, S., and Banerjee, R. P., influence of bile salts on the absorption of quinine, 266
Bauer, C. W., and Miskinis, A. M., the carboxypeptidase activity of Pancreatin U. S. P., 123
—— and Raskauskas, A. J., a critical investigation of the amylolytic activity of pancreatin and extract of malt, 113
—— and White, A. K., a study of the tryptic activity of Pancreatin U. S. P., 119
—— and Wilson, E. E., the lipolytic activity of Pancreatin U. S. P., 109
Beal, G. D., *see* Waters, K. L.
——, Waters, K. L., and Block, P., Jr., stability of iodine solutions and tinctures, 203
Beath, O. A., *see* Rosenfeld, I.
Bell, F. K., and Carr, C. J., the preparation and properties of the perchlorates of some choline esters, 272
Block, P., Jr., *see* Beal, G. D.
Braun, H. A., and Siegfried, A., the assay of digitalis. V. The guinea-pig method, 363
Brewer, J. H., and Dunning, J. H. F., an *in vitro* and *in vivo* study of glass particles in ampules, 289
Burger, A., the preparation of 1-diethylamino - 2 - (2,4,6 - triiodophenoxy) - ethane hydrochloride, 372
Burlage, H. M., pharmaceutical applications of isopropyl alcohol. II. Solubilities of local anesthetics, 16

C

Cahen, R. L., on the potency and toxicity of a new mercurial diuretic: sodium N-(β-hydroxymercurimethoxypropyl) - 2 - pyridone - 5-carboxylate, 139
Carol, J., and Molitor, J. C., a modified Kober method for the determination of α- and β-estradiol, 208
Carpenter, C. P., *see* Smyth, H. F., Jr.
Carr, C. J., *see* Bell, F. K.; Krantz, J. C., Jr.
Catlin, B. W., *see* Salle, A. J.
Chapman, C. W., Hager, G. P., and Shay, D. E., the *in vitro* antibacterial action of dialkylaminoalkyl ethers of stilbestrol and hexylresorcinol, 78
Christian, J. E., *see* Danielsson, I.; Magnusson, S.; Nelson, J. W.
Chou, T. Q., some minor alkaloids of Pei-Mu, *Fritillaria Roylei*, 215
——, Chu, J. H., and Mei, P. F., the sapogenins of the Chinese drug, Yüan Chih, *Polygala tenuifolia*, Willd., 241
Chu, J. H., *see* Chou, T. Q.
Clymer, H. A., and Ferlauto, R. J., availability of penicillin from various ointment bases, 211
Critchfield, F. H., *see* Shaffer, C. B.
Cronheim, G., organic ozonides as chemotherapeutic agents. I. Chemical studies, 274; II. Antiseptic properties, 278
—— and Wright, P. H., the colorimetric determination of bismuth in pharmaceutical preparations and in biological material, 62

D

Dandiya, P. C., *see* Basu, N. K.
Danielsson, I., Christian, J. E., and Jenkins, G. L., studies in chemotherapy. V. The synthesis of certain unsymmetrical disulfide derivatives the 4-nitrophenyl and the 4-aminophenyl radical, 261
David, N. A., *see* Kirchhof, A. C.
DeKay, H. G., *see* Wolff, J. E.
Dunning, J. H. F., *see* Brewer, J. H.
Dufrenoy, J., *see* Goyan, F. M.
—— and Goyan, F. M., a graphical calculator for statistical analysis, 309
Dutta, A. T., *see* Gupta, J. C.
—— and Ghosh, S., chemical examination of *Daemia extensa* R. Br. I., 250

E

Eble, T. E., *see* Fischbach, H.
Ederstrom, H. E., thyroid function and the duration of sodium pentobarbital anesthesia, 369
Edwards, L. D., *see* Haley, T. J.; Nelson, J. W.
Edwards, M. A., *see* Grubb, T. C.
Eisenberg, W. V., and Keenan, G. L., microscopic-crystallographic properties of crystalline sodium penicillin G, 294

F

Feinstein, L., and Scott, W. E., determination of nicotinic acid and riboflavin in multi-vitamin tablets, 342
Ferlauto, R. J., *see* Clymer, H. A.
Finkelstein, M., Gold, H., and Paterno, C. A., pharmacology of itaconic acid and its sodium, magnesium, and calcium salts, 173
Fischbach, H., Eble, T. E., and Mundell, M., the separation of penicillins by partition chromatography, 220
Fitzhugh, O. G., and Nelson, A. A., the comparative chronic toxicities of fumaric, tartaric, oxalic, and maleic acids, 217
Foley, E. J., and Lee, S. W., trimethyl cetyl ammonium pentachlorphenate (TCAP) and fatty acids as antifungal agents, 198
Foster, R. H. K., assay of heparin. I. Method and qualitative and quantitative aspects using beef and sheep plasmas, 243
Frediani, H. A., *see* Adams, J.
Friend, V. L., and Gold, H., a contribution to the pharmacology of succinic acid and its sodium and magnesium salts, 50

G

Georgi, C. E., pyrogen testing with "Polish" rabbits, 95
Ghosh, S., *see* Dutta, A. T.; Gupta, J. C.
Gibson, M. R., and Schwarting A. E., a spectrophotometric method of analysis of cascara sagrada preparations, 269
Giral, F., on the stability of vitamin C in aqueous solutions and in pharmaceutical preparations, 82
Gold, H., *see* Finkelstein, M.; Friend, V. L.
Golden, M. J., *see* Oster, K. A.
—— and Oster, K. A., studies on alcohol-soluble fungistatic and fungicidal compounds. II. Evaluation of a fungicidal laboratory test method, 359
Goldstein, S. W., barbiturates—are they narcotics? 97; barbiturates: a blessing and a menace, 5; a study of the modified Knudson–Dresbach colorimetric evaluation of digitalis: extraction studies on digitalis, 296
Gottlieb, S., chemical determination of synthetic estrogens. I. Basic data on the nitrosophenol reaction, 379
Goyan, F. M., *see* Dufrenoy, J.; Hind, H. W.
—— and Dufrenoy, J., a graphical calculator for bioassays, 305
——, Dufrenoy, J., Strait, L. A., Pratt, R., and Juntunen, T., a three-hour "physical development" cup-plate assay for penicillin, 65
Green, M. W., *see* Green, N.
Green, N., and Green, M. W., the assay of aspirin, acetophenetidin, and caffeine capsules, 235
Grote, I. W., *see* Holbert, J. M.
—— and Woods, M., studies on viburnum. III. The uterine sedative action of various fractions, 191
Grubb, T. C., and Edwards, M. A., the bactericidal action of oxygen-liberating substances on oral microorganisms. I. Studies with fusiform bacilli, 385
Gupta, J. C., Ghosh, S., Dutta, A. T., and Kahali, B. S., a note on the hypnotic principle of *Rauwolfia serpentina*, 416

H

Hager, G. P., *see* Chapman, C. W.
Haley, T. J., chemical differentiation of desoxyephedrine from other sympathomimetic amines, 301; desoxyephedrine—a review of the literature, 161
—— and Edwards, L. D., a study of the toxicity of two isomeric sodium hydroxymercuribenzoates, 30
——, Edwards, L. D., and Johnson, C. H., a pharmacological study of two isomeric sodium hydroxymercuribenzoates, 229
Halpern, A., *see* Jones, J. W.
—— and Zopf, L. C., hydrophilic properties of certain ointment base constituents, 101
Heller, G. L., and Kirch, E. R., the microbiological determination of proline, cystine, and isoleucine, 345
Hind, H. W., and Goyan, F. M., a new concept of the role of hydrogen ion concentration and buffer systems in the preparation of ophthalmic solutions, 33
——, Goyan, F. M., and Schwarz, T. W., notes on the role of hydrogen ion concentration and buffer systems in the preparation of ophthalmic solutions, 413
Holbert, J. M., *see* Krantz, J. C., Jr.
——, Noble, N., and Grote, I. W., a study of antacid buffers: I. The time factor in neutralization of gastric acidity, 149

I

Iwamoto, H. K., *see* Krantz, J. C., Jr.

J

James, A. E., Laquer, F. O., and McIntyre, J. D., a photoelectric colorimetric assay for digitoxin by comparison with a standard powder, 1
Jenkins, G. L., *see* Danielsson, I.; Magnusson, S.; Nelson, J. W.; Wolff, J. E.
Johnson, C. H., *see* Haley, T. J.
Jones, J. W., and Halpern, A., the synthesis and study of some N-dihydroxypropyl derivatives, 376
Jorgensen, P. S., comparison of several methods for the determination of arsenic in pentavalent arsenicals alone and in combination with bismuth, 27
Juntunen, T., *see* Goyan, F. M.

K

Kahali, B. S., *see* Gupta, J. C.
Keenan, G. L., microscopic description of the antihistamine substances benadryl and pyribenzamine, 281; *see* Eisenberg, W. V.
Kelly, R. J., *see* Waters, K. L.
Kirch, E. R., *see* Heller, G. L.
Kirchhof, A. C., David, N. A., Phatek, N. M., and Racely, C. A., further studies on two new lysergic acid compounds: *d*-lysergic acid-*dl*-hydroxybutylamide-2 (methergine) and dihydroergotamine (D.H.E. 45), 145
Kivela, E. W., *see* Lawrence, C. A.
Krantz, J. C., Jr., Holbert, J. M., Iwamoto, H. K., and Carr, C. J., sodium theophylline glycinate, 248
Kunze, F. M., *see* Laug, E. P.
Kwartler, C. E., *see* Lawrence, C. A.

L

Laquer, F. O., *see* James, A. E.
Laug, E. P., Vos, E. A., and Kunze, F. M., biologic assay of mercury ointments, 14
Lawrence, C. A., Kwartler, C. E., Wilson, V. L., and Kivela, E. W., germicidal action of some benzyl quaternary ammonium compounds having substituents in the aromatic nucleus, 353
Lee, S. W., *see* Foley, E. J.
Leva, E., *see* Snyder, F. H.

M

McIntyre, J. D., *see* James, A. E.
Mackowiak, E. J., and Barnard, R. D., comparative *in vitro* neutralization of heparin, 383
Magnusson, S., Christian, J. E., and Jenkins, G. L., studies in chemotherapy. IV. The synthesis of certain unsymmetrical disulfide derivatives of the 4-hydroxyphenyl radical, 257
Main, R. J., the nitrofurans—a new type of antibacterial agent, 317
Martin, G. J., toxicity of tyrosine in normal and vitamin-deficient animals, 187
Mei, P. F., *see* Chou, T. Q.
Miskinis, A. M., *see* Bauer, C. W.
Molinas, S., and Welch, H., the stability of commercial crystalline sodium penicillin in solution, 41
Molitor, J. C., *see* Carol, J.
Moore, A. W., *see* Tice, L. F.
Mukherjee, S., *see* Basu, U. P.
—— and Banerjee, R. P., the solubilization of quinine by bile salts, 314
Mundell, M., *see* Fischbach, H.

N

Nelson, A. A., *see* Fitzhugh, O. G.
Nelson, J. W., Edwards, L. D., Christian, J. E., and Jenkins, G. L., the study, testing, and synthesis of certain organic compounds for rodenticidal activity, 349
Noble, N., *see* Holbert, J. M.

O

Oberst, F. W., *see* Snyder, F. H.
Oster, K. A., *see* Golden, M. J.
—— and Golden, M. J., studies on alcohol-soluble fungistatic and fungicidal compounds. I. Evaluation of fungistatic laboratory test methods, 283
Ott, W. H., quantitative assay of the intravenous toxicity of streptomycin in mice, 193

P

Paterno, C. A., *see* Finkelstein, M.
Phatek, N. M., *see* Kirchhof, A. C.
—— and Saxey, E., effect of sodium 5-allyl - 5(methylbutyl)barbiturate (sodium seconal) on oxygen consumption in rats, 105
Prasad, S., influence of time of application of fertilizers on growth and alkaloidal content of *Hyoscyamus niger* Linn., 180; pharmacognostical studies of Brahmi, 393
Pratt, R., influence of phosphate on stability of partially purified penicillins, 69; *see* Goyan, F. M.
Price, C. W., *see* Randall, W. A.; Welch, H.
——, Randall, W. A., and Welch, H., the urinary excretion of penicillins G and K in rabbits, 19

R

Racely, C. A., *see* Kirchhoff, A. C.
Rahn, O., advisability of some proposed changes for the evaluation of disinfectants, 134
Randall, W. A., *see* Price, C. W.; Welch, H.
——, Welch, H., and Price, C. W., the effect of serum and organ emulsions on penicillin fractions, 17
Raskauskas, A. J., *see* Bauer, C. W.
Robinson, E. D., and Werch, S. C., the determination of acetanilid in the presence of caffeine, 185; the determination of camphor in solution with phenol in a high percentage of light mineral oil, 264
Rosenfeld, L. and Beath, O. A., pharmacological action of *Senecio riddellii*, 331

S

Salle, A. J., and Catlin, B. W., profile evaluations of germicides, 129
Saxey, E., *see* Phatek, N. M.
Schneiderwirth, H. J., and Wilcox, P. W., thixotropic mineral gels and their therapeutic possibilities, 402
Schwarting, A. E., *see* Gibson, M. R.
Schwarz, T. W., *see* Hind, H. W.
Scott, W. E., *see* Feinstein, L.
Shaffer, C. B., *see* Smyth, H. F., Jr.
—— and Critchfield, F. H., the absorption and excretion of the solid polyethylene glycols ("Carbowax" compounds), 152
Shay, D. E., *see* Chapman, C. W.
Siegfried, A., *see* Braun, H. A.
Smith, R. G., report on a collaborative study of the assay of ergot, 321
Smyth, H. F., Jr., Carpenter, C. P., and Shaffer, C. B., the toxicity of high molecular weight polyethylene glycols; chronic oral and parenteral administration, 157
——, Carpenter, C. P., and Weil, C. S., the chronic toxicity of hydroxyethyl cellulose for rats, 335
Snyder, F. H., Leva, E., and Oberst, F. W., an evaluation of adrenochrome and iodoadrenochrome based on blood sugar level in rabbits, 253
Spencer, D. A., *see* Ward, J. C.
Stone, K. G., the estimation of aloin in pharmaceuticals, 391
Strait, L. A., *see* Goyan, F. M.
Sumerford, W. T., chlorosulfonic acid in the synthesis of DDT and its *p*-halogen analogues, 127

T

Tice, L. F., and Moore, A. W., quaternary ammonium compounds as preservatives, 48

V

Vos, E. A., *see* Laug, E. P.

W

Ward, J. C., and Spencer, D. A., notes on the pharmacology of sodium fluoroacetate—compound 1080, 59
Waters, K. L., *see* Beal, G. D.
——, Kelly, R. J., and Beal, G. D., the determination of arsenic in official arsenicals, 26
Weil, C. S., *see* Smyth, H. F., Jr.
Welch, H., *see* Molinas, S.; Price, C. W.; Randall, W. A.
——, Price, C. W., and Randall, W. A., the effect of renal ligation on serum penicillin levels produced by penicillins G and K in rabbits, 23
——, Randall, W. A., and Price, C. W., the demonstration in amorphous penicillin of a non-penicillin factor which enhances its protective action, 337
Welsh, L. H., the use of acetylation in the quantitative separation of ephedrine from procaine, 373
Werch, S. C., *see* Robinson, E. D.
White, A. K., *see* Bauer, C. W.
Wilcox, P. W., *see* Schneiderwirth, H. J.
Wilson, E. E., *see* Bauer, C. W.
Wilson, V. L., *see* Lawrence, C. A.
Wirth, E. H., *see* Youngken, H. W.
Wolff, J. E., DeKay, H. G., and Jenkins, G. L., lubricants in compressed tablet manufacture, 407
Wood, M., *see* Grote, I. W.
Wright, P. H., *see* Cronheim, G.

Y

Youngken, H. W., and Wirth, E. H., pharmacognostical studies of four American arnicas, 85

Z

Zopf, L. C., *see* Halpern, A.

SUBJECT INDEX

A

Absorption and excretion of the solid polyethylene glycols ("Carbowax" compounds), 152
of quinine, influence of bile salts on the, 266
Acetanilid, the determination of, in the presence of caffeine, 185
Acetophenetidin, aspirin, and caffeine capsules, the assay of, 235
Acetylation, the use of, in the quantitative separation of ephedrine from procaine, 373
Adrenochrome and iodoadrenochrome, an evaluation of, based on blood sugar level in rabbits, 253
Alcohol, *see also* Isopropyl alcohol
-soluble fungistatic and fungicidal compounds, studies on. I. Evaluation of fungistatic laboratory test methods, 283; II. Evaluation of a fungicidal laboratory test method, 359
Alkaloidal content and growth of *Hyoscyamus niger* Linn., influence of time of application of fertilizers on, 180
Alkaloids, some minor, of Pei-Mu, *Fritallaria Roylei*, 215
Aloin, the estimation of, in pharmaceuticals, 391
1 - Amino - 8 - naphthol - 2,4 - disulfonic acid, synthesis of iodosulfophenylazo and iodocarboxyphenylazo derivatives, of, 76
4-Aminophenyl radical and the 4-nitrophenyl radical, the synthesis of certain unsymmetrical disulfide derivatives of. V. Studies in chemotherapy, 261
Ammonium compounds, quaternary, as preservatives, 48
some benzyl quaternary, germicidal action of, having substituents in the aromatic nucleus, 353
Amorphous penicillin, the demonstration in, of a non-penicillin factor which enhances its protective action, 337
Ampules, an *in vitro* and *in vivo* study of glass particles in, 289
Amylolytic activity of pancreatin and extract of malt, a critical investigation of the, 113
Anesthetics, local, solubilities of, II. Pharmaceutical applications of isopropyl alcohol, 16
Antacid buffers, a study of: I. The time factor in neutralization of gastric acidity, 149
Antibacterial action, *in vitro*, of dialkylaminoalkyl ethers of stilbestrol and hexylresorcinol, 78
Antibacterial agent, a new type of—the nitrofurans, 317
Antifungal agents, trimethyl cetyl ammonium pentachlorphenate (TCAP) and fatty acids as, 198
Antihistamine substances benadryl and pyribenzamine, microscopic description of the, 281
Antiseptic properties, II. Organic ozonides as chemotherapeutic agents, 278
Aqueous solutions, on the stability of vitamin C in, and in pharmaceutical preparations, 82
Arnicas, pharmacognostical studies of four American, 85
Arsenic, comparison of several methods for the determination of, in pentavalent arsenicals alone and in combination with bismuth, 27
the determination of, in official arsenicals, 26
Arsenicals, official, the determination of arsenic, in, 26
pentavalent, comparison of several methods for the determination of arsenic in, alone and in combination with bismuth, 27
Ascorbic acid, iodimetric titrimetric determination of, 170
Aspirin, acetophenetidin, and caffeine capsules, the assay of, 235

B

Bactericidal action of oxygen-liberating substances on oral microorganisms. I. Studies with fusiform bacilli, 385
Barbiturates—are they narcotics? 97
a blessing and a menace, 5
Benadryl and pyribenzamine, antihistamine substances, microscopic description of the, 281
Benzyl quaternary ammonium compounds, germicidal action of some, having substituents in the aromatic nucleus, 353
Bile salts, influence of, on the absorption of quinine, 266
the solubilization of quinine by, 314
Bioassays, graphical calculator for, 305
Biologic assay of mercury ointments, 14
Biological material, colorimetric determination of bismuth in, and in pharmaceutical preparations, 62
Bismuth, colorimetric determination of, in pharmaceutical preparations and in biological material, 62
comparison of several methods for the determination of arsenic in pentavalent arsenicals alone and in combination with, 27
Blood coagulation, the effect of saccharin ingestion on, and the *in vitro* anticoagulant effect of saccharin and of ferriheme, 225
Book reviews
Advancing Fronts in Chemistry. Volume II, Chemotherapy, 128
Bacterial Chemistry and Physiology, 192
Bacteriology—Laboratory Directions for Pharmacy Students, 320
Biochemistry of Cancer, 256
Chemistry of Food and Nutrition, 128
Ether for Anesthesia, 160
Inorganic Chemistry, 401
Limit Tests for Impurities, 160
Penicillin: Its Properties, Uses, and Preparations, 96
The Pharmaceutical Industry, 256
Physical Constants of Hydrocarbons. Volume IV. Polynuclear Aromatic Hydrocarbons, 223
Practical Physiological Chemistry, 255
Qualitative Analysis by Spot Tests, 128
Vitamins and Hormones, Volume IV, 288
Volumetric Analysis. Vol. II. Titration Methods, 368
Brahmi, pharmacognostical studies of, 393
Buffers, antacids, *see* Antacid buffers
Buffer systems and hydrogen ion concentration, a new concept of the role of, in the preparation of ophthalmic solutions, 33; notes on, 413

C

Caffeine, aspirin, and acetophenetidin capsules, the assay of, 235
the determination of acetanilid in the presence of, 185
Calcium salts, sodium salts, and magnesium salts of itaconic acid, pharmacology of, 173
Calculator, *see* Graphical calculator
Camphor, the determination of, in solution with phenol in a high percentage of light mineral oil, 264
"Carbowax" compounds (solid polyethylene glycols), the absorption and excretion of the, 152
Carboxypeptidase activity of Pancreatin U. S. P., 123
Cascara Sagrada preparations, spectrophotometric method of analysis of, 269
Cellulose, hydroxyethyl, the chronic toxicity of, for rats, 335
Central nervous system stimulants, a note on the synergism of the, 414
Chemotherapeutic agents, organic ozonides as. I. Chemical studies, 274; II. Antiseptic properties, 278
Chemotherapy, studies in. IV. The synthesis of certain unsymmetrical disulfide derivatives of the 4-hydroxyphenyl radical, 257; V. The synthesis of certain unsymmetrical disulfide derivatives of the 4-nitrophenyl and the 4-aminophenyl radical, 261
Chlorosulfonic acid in the synthesis of DDT and its *p*-halogen analogues, 127
Choline esters, the preparation and properties of the perchlorates of some, 272
Colorimetric assay, photoelectric, for digitoxin by comparison with a standard powder, 1
Colorimetric determination of bismuth in pharmaceutical preparations and in biological material, 62
Color reaction, a simple, for piperazine, 224
Compound 1080—sodium fluoroacetate, notes on the pharmacology of, 59
Cystine, proline, and isoleucine, microbiological determination of, 345

D

Daemia extensa R. Br. I., chemical examination of, 250
DDT, chlorosulfonic acid in the synthesis of, and its *p*-halogen analogues, 127
Desoxyephedrine, chemical differentiation of, from other sympathomimetic amines, 301
a review of the literature, 161
D.H.E. 45, *see* Lysergic acid compounds
Dialkylaminoalkyl ethers of stilbestrol and hexylresorcinol, the *in vitro* antibacterial action of, 78
1 - Diethylamino - 2 - (2,4,6 - triiodophenoxy) ethane hydrochloride, the preparation of, 372
Digitalis, the assay of. V. The guinea-pig method, 363
a study of the modified Knudson–Dresbach colorimetric evaluation of: extraction studies on digitalis, 296
Digitoxin, a photoelectric colorimetric assay for, by comparison with a standard powder, 1
Dihydroergotamine (D.H.E. 45), *see* Lysergic acid compounds
N-Dihydroxypropyl derivatives, the synthesis and study of some, 376
Disinfectants, advisability of some proposed changes for the evaluation of, 134
Disulfide derivatives of the 4-hydroxyphenyl radical, the synthesis of certain unsymmetrical, IV. Studies in chemotherapy, 257
of the 4-nitrophenyl and the 4-aminophenyl radical, the synthesis of certain unsymmetrical, V. Studies in chemotherapy, 257
Drug, Chinese, Yüan Chih, *Polygala tenuifolia*, Willd., sapogenins of the, 241

E

Ephedrine, the use of acetylation in the quantitative separation of, from procaine, 373
Ergot, report on a collaborative study of the assay of, 321
Estradiol, α- and β-, a modified Kober method for the determination of, 208
Estrogens, chemical determination of synthetic. I. Basic data on the nitrosophenol reaction, 379
Excretion and absorption of the solid polyethylene glycols ("Carbowax" compounds), 152

F

Fatty acids and trimethyl cetyl ammonium pentachlorphenate (TCAP) as antifungal agents, 198
Ferriheme, the effect of saccharin ingestion on blood coagulation and the *in vitro* anticoagulant effect of saccharin and, 225
Fertilizers, influence of time of application of, on growth and alkaloidal content of *Hyoscyamus niger* Linn., 180
Fritallaria Roylei, Pei-Mu, some minor alkaloids of, 215
Fumaric acid, tartaric, oxalic, and maleic acids, the comparative chronic toxicities of, 217

Fungicidal compounds, and fungistatic, studies on alcohol-soluble. I. Evaluation of fungistatic laboratory test methods, 283; II. 359
Fungistatic compounds, *see* Fungicidal compounds
Fusiform bacilli, studies with, I. The bactericidal action of oxygen-liberating substances on oral microorganisms, 385

G

Gastric acidity, the time factor in neutralization of, I. A study of antacid buffers, 149
Gels, thixotropic mineral, and their therapeutic possibilities, 402
Germicidal action of some benzyl quaternary ammonium compounds having substituents in the aromatic nucleus, 353
Germicides, profile evaluations of, 129
Glass particles in ampules, an *in vitro* and *in vivo* study of, 289
Graphical calculator for bioassays, 305; for statistical analysis, 309
Growth and alkaloidal content of *Hyoscyamus niger* Linn., influence of time of application of fertilizers on, 180
Guinea-pig method, V. The assay of digitalis, 363

H

Heparin, assay of. I. Method and qualitative and quantitative aspects using beef and sheep plasmas, 243
comparative *in vitro* neutralization of, 383
Hexylresorcinol and stilbestrol, the *in vitro* antibacterial action of dialkylaminoalkyl ethers of, 78
Hydrogen ion concentration and buffer systems, a new concept of the role of, in the preparation of ophthalmic solutions, 33; notes on, 413
Hydrophilic properties of certain ointment base constituents, 101
Hydroxyethyl cellulose, the chronic toxicity of, for rats, 335
4-Hydroxyphenyl radical, the synthesis of certain unsymmetrical disulfide derivatives of the, IV. Studies in chemotherapy, 257
Hyoscyamus niger Linn., influence of time of application of fertilizers on growth and alkaloidal content of, 180
Hypnotic principle of *Rauwolfia serpentina,* a note on the, 416

I

Iodimetric titrimetric determination of ascorbic acid, 170
Iodine Research Award nominations requested, 358, 406
Iodine solutions and tinctures, stability of, 203
Iodoadrenochrome and adrenochrome, an evaluation of, based on blood sugar level in rabbits, 253
Iodosulfophenylazo and iodocarboxyphenylazo derivatives of 1-amino-8-naphthol-2,4-disulfonic acid, synthesis of, 76
Isoleucine, cystine, and proline, microbiological determination of, 345
Isopropyl alcohol, pharmaceutical applications of. II. Solubilities of local anesthetics, 16
Itaconic acid and its sodium, magnesium, and calcium salts, pharmacology of, 173

K

Knudson-Dresbach colorimetric evaluation of digitalis, a study of the modified: extraction studies on digitalis, 296
Kober method, a modified, for the determination of α- and β-estradiol, 208

L

Laxative action of succinates in man, 56
Light mineral oil, *see* Mineral oil
Lipolytic activity of Pancreatin U. S. P., 109
Local anesthetics, *see* Anesthetics
Lubricants in compressed tablet manufacture, 407
d-**Lysergic acid**-*dl*-**hydroxybutylamide-2,** *see* Lysergic acid compounds
Lysergic acid compounds, further studies on two new: *d*-lysergic acid-*dl*-hydroxybutylamide-2 (methergine) and dihydroergotamine (D.H.E. 45), 145

M

Magnesium salts, sodium salts, and calcium salts of itaconic acid, pharmacology of, 173
and sodium salts, a contribution to the pharmacology of succinic acid and its, 50
Maleic acid, oxalic, fumaric, and tartaric acids, the comparative chronic toxicities of, 217
Malt, extract of, and pancreatin, a critical investigation of the amylolytic activity of, 113
Mercurial diuretic, potency and toxicity of a new: sodium N-(β-hydroxymercuri - γ - methoxypropyl) - 2-pyridone-5-carboxylate, 139
Mercury ointments, biologic assay of, 14
Methergine, *see* Lysergic acid compounds
Microbiological determination of proline, cystine, and isoleucine, 345
Microorganisms, oral, bactericidal action of oxygen-liberating substances on. I. Studies with fusiform bacilli, 385
Microscopic-crystallographic properties of crystalline sodium penicillin G, 294
Microscopic description of the antihistamine substances benadryl and pyribenzamine, 281
Mineral oil, light, the determination of camphor in solution with phenol in a high percentage of, 264

N

Neutralization, comparative *in vitro,* of heparin, 383
Nicotinic acid and riboflavin, determination of, in multi-vitamin tablets, 342
Nitrofurans, the—a new type of antibacterial agent, 317
4-Nitrophenyl radical and the 4-aminophenyl radical, the synthesis of certain unsymmetrical disulfide derivatives of, V. Studies in chemotherapy, 261
Nitrosophenol reaction, basic data on the, I. Chemical determination of synthetic estrogens, 379

O

Official arsenicals, *see* Arsenicals
Ointment base constituents, hydrophilic properties of certain, 101
Ointment bases, availability of penicillin from various, 211
Ointments, mercury, biologic assay of, 14
Ophthalmic solutions, a new concept of the role of hydrogen ion concentration and buffer systems in the preparation of, 33; notes on, 413
Organic compounds, the study, testing, and synthesis of certain, for rodenticidal activity, 349
Organic ozonides as chemotherapeutic agents. I. Chemical studies, 274; II. Antiseptic properties, 278
Organ and serum emulsions, the effect of, on penicillin fractions, 17
Oxalic acid, fumaric, tartaric, and maleic acids, the comparative chronic toxicities of, 217
Oxygen consumption, effect of sodium 5-allyl-5(methylbutyl)barbiturate (sodium seconal) on, in rats, 105
Oxygen-liberating substances, bactericidal action of, on oral microorganisms. I. Studies with fusiform bacilli, 385
Oxytocic activity of pitocin and pitressin on isolated segments of mammalian uteri by a graded response method, 73
Ozonides, *see* Organic ozonides

P

Pancreatin and extract of malt, a critical investigation of the amylolytic activity of, 113
Pancreatin U. S. P., the carboxypeptidase activity of, 123
lipolytic activity of, 109
a study of the tryptic activity of, 119
Partition chromatography, the separation of penicillins by, 220
Pei-Mu, *Fritallaria Roylei,* some minor alkaloids of, 215
Penicillin, amorphous, the demonstration in, of a non-penicillin factor which enhances its protective action, 337
availability of, from various ointment bases, 211
commercial crystalline sodium, the stability of, in solution, 41
fractions, the effect of serum and organ emulsions on, 17
levels, serum, the effect of renal ligation on, produced by penicillins G and K in rabbits, 23
a three-hour "physical development" cup-plate assay for, 65
Penicillin G, *see also* Penicillins G and K crystalline sodium, microscopic-crystallographic properties of, 294
Penicillins, influence of phosphate on stability of partially purified, 69
the separation of, by partition chromatography, 220
Penicillins G and K, the effect of renal ligation on serum penicillin levels produced by, in rabbits, 23
urinary excretion of, in rabbits, 19
Pentavalent arsenicals, *see under* Arsenicals
Pentobarbital sodium anesthesia, the duration of, and thyroid function, 369
Perchlorates of some choline esters, the preparation and properties of the, 272
pH, *see* Hydrogen ion concentration
Pharmaceutical applications of isopropyl alcohol. II. Solubilities of local anesthetics, 16
Pharmaceutical preparations, colorimetric determination of bismuth in, and in biological material, 62
on the stability of vitamin C in aqueous solutions and in, 82
Pharmaceuticals, the estimation of aloin in, 391
Pharmacognostical studies of Brahmi, 393
of four American arnicas, 85
Pharmacological action of *Senecio riddellii,* 331
Pharmacology of itaconic acid and its sodium, magnesium, and calcium salts, 173
Phenol, the determination of camphor in solution with, in a high percentage of light mineral oil, 264
Phosphate, influence of, on stability of partially purified penicillins, 69
Photoelectric colorimetric assay for digitoxin by comparison with a standard powder, I
"Physical development" cup-plate assay for penicillin, a three-hour, 65
Piperazine, a simple color reaction for, 224
Pitocin and pitressin, on the oxytocic activity of, on isolated segments of mammalian uteri by a graded response method, 73
Pitressin, *see* Pitocin
Plasmas, beef and sheep, method and qualitative and quantitative aspects using, I. Assay of heparin, 243
Polyethylene glycols, solid ("Carbowax" compounds), the absorption and excretion of the, 152
toxicity of high molecular weight; chronic oral and parenteral administration, 157
Polygala tenuifolia, Willd., Yüan Chih, sapogenins of the Chinese drug, 241
Potency and toxicity of a new mercurial diuretic: sodium N-(β-hydroxymercuri - γ - methoxypropyl) - 2 - pyridone-5-carboxylate, 139
Premna integrifolia Linn., chemical investigation of, 389
Preservatives, quaternary ammonium compounds as, 48
Procaine, the use of acetylation in the quantitative separation of ephedrine from, 373
Profile evaluations of germicides, 129
Proline, cystine, and isoleucine, microbiological determination of, 345
Pyribenzamine and benadryl, antihistamine substances, microscopic description of the, 281

Pyrogen testing with "Polish" rabbits, 95

Q

Quantitative assay of the intravenous toxicity of streptomycin in mice, 193
Quaternary ammonium compounds, benzyl, germicidal action of some, having substituents in the aromatic nucleus, 353
as preservatives, 48
Quinine, influence of bile salts on the absorption of, 266
the solubilization of, by bile salts, 314

R

Raufolia serpentina, a note on the hypnotic principle of, 416
Renal ligation, the effect of, on serum penicillin levels produced by penicillins G and K in rabbits, 23
Riboflavin and nicotinic acid, determination of, in multi-vitamin tablets, 342
Rodenticidal activity, the study, testing, and synthesis of certain organic compounds for, 349

S

Saccharin ingestion, the effect of, on blood coagulation and the *in vitro* anticoagulant effect of saccharin and of ferriheme, 225
Sapogenins of the Chinese drug, Yüan Chih, *Polygala tenuifolia*, Willd., 241
Seconal, sodium, *see* Sodium 5-allyl-5(methylbutyl) barbiturate
Senecio riddellii, pharmacological action of, 331
Serum and organ emulsions, the effect of, on penicillin fractions, 17
penicillin levels, the effect of renal ligation on, produced by penicillins G and K in rabbits, 23
Sodium 5-allyl-5(methylbutyl) barbiturate (sodium seconal) on oxygen consumption in rats, 105
Sodium fluoroacetate—compound 1080, notes on the pharmacology of, 59
Sodium hydroxymercuribenzoates, isomeric, a pharmacological study of two, 229
isomeric, a study of the toxicity of two, 30
Sodium N - (β - hydroxymercuri - γ - methoxypropyl) - 2 - pyridone - 5-carboxylate, on the potency and toxicity of a new mercurial diuretic, 139
Sodium penicillin, commercial crystalline, the stability of, in solution, 41
Sodium salts, magnesium salts, and calcium salts of itaconic acid, pharmacology of, 173
and magnesium salts, a contribution to the pharmacology of succinic acid and its, 50
Sodium theophylline glycinate, 248
Solubilization of quinine by bile salts, 314
Solutions, *see* Ophthalmic solutions; Iodine solutions
Spectrophotometric method of analysis of Cascara Sagrada preparations, 269
Stability of iodine solutions and tinctures, 203
Standard powder, a photoelectric colorimetric assay for digitoxin by comparison with a, 1
Statistical analysis, a graphical calculator for, 309
Stilbestrol and hexylresorcinol, the *in vitro* antibacterial action of dialkylaminoalkyl ethers of, 78
Stimulants, central nervous system, a note on the synergism of the, 414
Streptomycin, quantitative assay of the intravenous toxicity of, in mice, 193
Succinates, laxative action of, in man, 56
Succinic acid and its sodium and magnesium salts, a contribution to the pharmacology of, 50
Sympathomimetic amines, chemical differentiation of desoxyephedrine from other, 301
Synergism of the central nervous system stimulants, a note on the, 414

T

Tablet manufacture, compressed, lubricants in, 407
Tablets, multi-vitamin, determination of nicotinic acid and riboflavin in, 342
Tartaric acid, fumaric, oxalic, and maleic acids, the comparative chronic toxicities of, 217
TCAP, *see* Trimethyl cetyl ammonium pentachlorphenate
Theophylline sodium glycinate, 248
Therapeutic possibilities, thixotropic mineral gels and their, 402
Thixotropic mineral gels and their therapeutic possibilities, 402
Thyroid function and the duration of sodium pentobarbital anesthesia, 369
Toxicities, the comparative chronic, of fumaric, tartaric, oxalic, and maleic acids, 217
Toxicity, chronic, of hydroxyethyl cellulose, for rats, 335
of high molecular weight polyethylene glycols; chronic oral and parenteral administration, 157
intravenous, of streptomycin in mice, quantitative assay of the, 193
and potency of a new mercurial diuretic: sodium N-(β-hydroxymercuri - γ - methoxypropyl) - 2-pyridone-5-carboxylate, 139
of two isomeric sodium hydroxymercuribenzoates, a study of the, 30
of tyrosine in normal and vitamin-deficient animals, 187
Trimethyl cetyl ammonium pentachlorphenate (TCAP) and fatty acids as antifungal agents, 198
Tryptic activity of Pancreatin U. S. P., a study of the, 119
Tyrosine, toxicity of, in normal and vitamin-deficient animals, 187

U

United States Pharmacopœial Convention, seventh annual financial report, 410
Urinary excretion of penicillins G and K in rabbits, 19
Uterine sedative action of various fractions, III. Studies on viburnum, 191

V

Viburnum, studies on. III. The uterine sedative action of various fractions, 191
Vitamin C, on the stability of, in aqueous solutions and in pharmaceutical preparations, 82
Vitamin-deficient animals, and normal, toxicity of tyrosine in, 187
Vitamin tablets, multi-, determination of nicotinic acid and riboflavin in, 342

Y

Yüan Chih, *Polygala tenuifolia*, Willd., sapogenins of the Chinese drug, 241